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VETERINARY REVIEWS AND ANNOTATIONS

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COMMONWEALTH BUREAU OF ANIMAL HEALTH

VETERINARY REVIEWS AND ANNOTATIONS

Volume 7 **October, 1961** *Part 2*

Microbiological Aspects of *Listeria monocytogenes* with special reference to Listeriosis in Animals

BY

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Listeria is a genus of the Family Corynebacteriaceae in the Order Eubacteriales. Members of the Corynebacteriaceae are, characteristically, rod-shaped organisms found in angular or picket formation due to their snapping division; Gram-positive but frequently Gram-negative when old; and do not actively ferment sugars anaerobically. The genus Listeria is further differentiated as being pathogenic to animals including man, and causing a monocytosis. There is only one species, L. monocytogenes.

Description of Listeria monocytogenes. The rods are small (0.4–0.5 x 0.5–2.0 μ), with rounded ends, and sometimes appear slightly curved. They usually occur singly, in parallel pairs, or in a V formation. Pleomorphism is shown occasionally. The organisms grow well on ordinary media, although better if some blood is included, and are aerobic to micro-aerophilic.

L. monocytogenes has a maximum of four flagella at room temperature (21°C.) with a tendency to tri-, bi-, and mono-flagellate forms at 37°C. In the third form the flagellum is attached sub-terminally (Griffin & Robbins, 1944a). Leifson & Palen (1955) substantiated Griffin & Robbins' findings and in detailed studies distinguished "normal" strains and seven variant forms. The motility shown has been aptly described as "tumbling" (Zink et al., 1951).

Jaeger & Myers (1954) observed a number of morphological changes occurring at low temperature. These were:- an increase in length, a

great amount of palisading and parallel formation, granulation, and short chain formation. At 2.8–5.0°C. a capsule-like material surrounds the cell and this is not apparent at 37°C.

Colonial forms have been studied by Gray (1957) and Gray, Stafseth & Thorp (1957) who recommended the use of a binocular scanning microscope and oblique direct light for the detection of L. monocytogenes colonies on tryptose agar plates. They found that colonies were round; slightly raised with a finely-textured surface; entire margin; bluish-green; and of a watery consistency. They distinguished twenty different colonial forms. Hutner (1942) found that excess acid caused change of colony form from smooth to rough.

Little work has been done on the growth factor requirements of L. monocytogenes. Porter & Pelczar (1941) found that most strains required riboflavin, biotin and haemin. A few could grow with the addition of riboflavin alone although they gave inconsistent results. Thiamin, inositol and glutamine stimulated growth. Hutner (1942) substantiated these findings but pointed out that there was a growth factor present in the "vitamin-free" casein used by himself and Porter. Kodicek & Worden (1944) found that the inhibitory effect of linoleic acid was reversed by cholesterol, lecithin, lumisterol, calciferol, alpha-tocopherol and alpha-tocopherol acetate.

A number of indicator media have been suggested by various workers. Sohier (1948) described an aesculin medium which becomes black in the presence of L. monocytogenes. Gray, Stafseth & Thorp (1950), Olson, Dunn & Rollins (1953), and Shimizu et al. (1954) advocated the use of potassium tellurite to which L. monocytogenes is fairly tolerant. A sucrose medium was described by Kröger (1955), and Hartwigk (1958) recommends a medium of 0.3% agar and 1% glucose through which L. monocytogenes shows a typical swarming.

Nomenclature. In 1926, Murray et al. reported a disease in their laboratory rabbits and guinea-pigs. Young rabbits chiefly were involved. Small, motile, round-ended, Gram-positive, non spore-forming rods were isolated from the liver, spleen, myocardium and mesenteric lymph nodes. Injection of a sub-lethal dose of the organism gave rise to a marked circulatory monocytosis in rabbits. The name Bacterium monocytogenes was proposed for the organism. In 1927, Pirie investigated a disease of gerbils on the veld of South Africa and thought the causative organism isolated to be a new species and proposed the name Listerella hepatolytica (in honour of Lord Lister), but when the organism proved to be the same as Bact. monocytogenes, the name Listerella monocytogenes was adopted. However, the generic name Listerella was not accepted by the Nomenclature Committee of the International Congress for Microbiology (Becker, 1951) as it was already in use for a Mycetozoan.

In 1940, Pirie suggested the generic name Listeria and this appears in Bergey's Manual (1957). But this name is also given to a certain plant group (Graham, Levine & Morrill, 1943). Wilson & Miles (1955) consider that the differences between Erysipelothrix and Listeria are of specific rather than generic rank and refer to E. monocytogenes. However,

Listeria causes a different disease than Erysipelothrix and animals are not equally susceptible to both. The organisms are antigenically different and Listeria is motile. These differences seem too great to be characteristic of a species difference only. Thus there is a state of indecision, some referring to E. monocytogenes, some to L. monocytogenes but all use listeriosis as the name of the disease.

Although Murray et al. (1926) are credited with being the first to note this organism it seems likely that the Swedish worker, Hülphers, may have been. In 1911 he found islets of necrosis in the liver of rabbits caused by small Gram-positive rods which he called Bacterium hepatica (Lesbouyries, 1943). Goodpasture (1924) discussed a spontaneous encephalitis in rabbits due to a Gram-positive organism and characterized by a mononuclear exudate in the meninges. A number of other publications prior to 1926 refer to "diphtheroid organisms" which may possibly have been L. monocytogenes (Finegold et al., 1954).

The disease Listeriosis. Listeriosis is of world wide distribution, affects a wide variety of species and appears in many different forms. Meningo-encephalitis and/or abortion may be caused in man and farm animals while in rodents and fowls necrotic hepatitis and myocarditis are usually found. Listeriosis in man has been extensively reviewed by Seeliger & Cherry (1957), its relationship to glandular fever remains indefinite and the present situation is clarified by Hyslop & Osborne (1959).

Listeriosis in ruminants. There is a prodromal febrile phase with dulness and anorexia, followed in two to three days by signs of nervous derangement. The first signs are often missed. Infected animals wander aimlessly, then tend to turn in circles, always in the same direction, hence the name "circling disease". The infected animal may stand with its head pushed against some solid object. In a few cases there is mania, and salivation. Nasal discharge, or torticollis, are not uncommon. Eventually the animal falls, is unable to rise, becomes comatose and dies.

Abortion may occur usually in the fourth to eighth month of pregnancy in cattle, and in the later stages in sheep. In young animals there may be septicaemia with general weakness and wasting rapidly followed by death. L. monocytogenes has also been found in cases of mastitis in cattle.

Localized damage is found in the central nervous system (c.n.s.). Mild meningitis is often confined to the meninges which cover the cerebellum, medulla and anterior spinal cord. There may be excess cerebro-spinal fluid, and sections show pin-point haemorrhages or even smaller necrotic areas within the brain substance. Peri-vascular oedema may be seen with well-marked "cuffing" caused by mononuclear cell infiltration surrounding the small blood vessels of the brain and cord. These changes may also occur elsewhere in the c.n.s. Some degenerative areas show neutrophilic infiltration. Larger zones of complete necrosis may be wholly or partly infiltrated by lymphocytes, monocytes and polymorphic neutrophiles. Oedematous effusions associated with degenerative

changes in the liver and other parenchymatous organs may occur.

L. monocytogenes causes neonatal septicaemia in young lambs. Petechial haemorrhages on serous surfaces, and excess fluid within the body cavities may be found together with marked congestion of parenchymatous organs. The lymph nodes are swollen, haemorrhagic and oedematous. A well-established infection results in multiple small zones of hepatic necrosis. Lesions found in rodents resemble those found in young lambs.

Cattle.

Encephalitis. The first case was recorded in New Jersey (Jones & Little, 1934). In the following years the disease occurred in many other States of the U.S.A. and was recorded in Iowa (Biester & Schwarte, 1941b); Minnesota (Pomeroy et al., 1943); S.W. Virginia (Hatch, 1945); Pennsylvania (Boucher, 1946) Ohio (Cole, 1946); Michigan (Thorp et al., 1947); Colorado (Jensen & Mackey, 1949); and S. Georgia (Boyer, 1951). It was recorded in Canada by Fish & Schroeder (1949). Following the report from Harbour (1941) in N. Wales, there were no further cases recorded in Europe until one from the Netherlands (van der Schaaf et al., 1951). Then came reports from Russia (Stol'nikov, 1955, 1957), Germany (Hartwigk & Renk, 1956), Azerbaijan (Mamedov, 1957), Scotland (Dickie et al., 1958) and Italy (Badiali, 1958).

Abortion. Graham, Dunlap & Brandly (1938) in the U.S.A. first associated L. monocytogenes with abortion in cattle, and the organism was isolated from aborted foetuses by Graham, Hester & Levine (1940a); Ferguson et al. (1951) and by Olson (1945) who also found an associated streptococcal infection. Abortion due to listeriosis has also been recorded in Israel (Levi et al., 1952), Portugal (Moreira-Jacob, 1956), France (Goyon & Lecomte, 1957) and in Azerbaijan (Mamedov, 1957).

Mastitis. L. monocytogenes has been implicated at least three times in cases of mastitis, by Wramby (1944), de Vries & Willems (1957), and Hyslop & Osborne (1959).

Calves. The first bovine case in Gt. Britain was diagnosed by Harbour (1941), when he isolated L. monocytogenes from the liver, lungs, gall bladder and ulcers on the mucosa of the abomasum of a nine-day-old calf. He also noted necrotic foci on the liver. This latter feature has been investigated by Rubarth & Wollarz (1951) and Gray, Lassiter, Webster et al. (1956). Rubarth & Wollarz examined calf livers showing miliary foci from an abattoir in Norway and found that all were due to the presence of L. monocytogenes and not to Salmonella as might have been expected. Gray, Lassiter, Webster et al. found a clinical picture of general weakness and weight loss associated with the presence of L. monocytogenes in the liver of calves less than 19 days old. They consider that L. monocytogenes may play a greater role in death of young calves than is at present supposed.

An outbreak of the encephalitic form in which 8 of 27 beef calves died was recorded by Pounden et al. (1947).

Goats. The first records of listeriosis in goats were of the encephalitic type and came from the U.S.A. (King, 1940; Olafson, 1940).

Further reports then followed from various parts of the world: U.S.A. (Gifford & Eveleth, 1942; Kaplan & Lager, 1945; Gray, Millerick & Johnston, 1952) the Netherlands (Jansen & van der Hurk, 1943); Scandinavia (Naerland, 1950; Sandbu, 1956), Japan (Tajima, 1950, Asahi et al., 1953); Russia (Khalimbekov, 1955; Amanzhulov, 1953; Ivanov, 1957), and Egypt (Ramadan, 1954). In most cases L. monocytogenes caused encephalitis, although Amanzhulov (1953) and Sandbu (1956) reported abortion. Jansen & van der Hurk (1943) described a case of septicaemia in which there was inflammation and necrosis of the large intestine.

Sheep.

Encephalitis. There have been numerous reports of encephalitis or "circling disease" caused by L. monocytogenes infection, the first being reported by Gill (1931) in New Zealand. Since then reports of the disease have come from all parts of the world. These are tabulated (Table 1):

Table 1

Reports of the encephalitic form of listeriosis in sheep

Year	Country	Author	Notes
1931	New Zealand	Gill	
1932	Australia	Hindmarsh & Blumer	
1937	U.S.A.	Jungherr	"Listeria-like" organism isolated
1938	U.S.A.	Morin	Case with symptoms similar to those described by Jungherr (1937)
1938	U.S.A.	Graham, Dunlap & Brandly	Two case reports
1939	U.S.A.	Biester & Schwarte	Noted similarity to virus disease
1940	Germany	Pallaske	
1940	U.S.A.	Graham, Hester & Levine (1940b)	Outbreaks in 3 flocks
1941	U.S.A.	Henderson	
1941	U.S.A.	Jensen & Gay	
1941	U.S.A.	Hoffman	
1942	Norway	Grini	
1942	U.S.A.	Hoffman & Lenarz	

Year	Country	Author
1944	U.S.A.	Spencer et al.
1944	E. Prussia	Pothmann
1947	U.S.A.	Gray, Thorp, Nelson et al.
1948	U.S.A.	Ryff & Lee
1949	U.S.A.	Stoenner et al.
1949	U.S.A.	Jensen & Mackey
1950	India	Viswanathan & Ayyar
1950	Norway	Eieland & Finborud
1951	Japan	Sugawa
1952	Hungary	Mócsy & Sályi
1953	Australia	Clapp
1954	Egypt	Ramadan
1954	Japan	Hirato et al.
1954	Russia	Orobinski
1954	Norway	van Ulsen
1955	Hungary	Kemenes
1955	Poland	Dąbrowski & Meresta
1955	Russia	Tyvshnyakova
1955	Russia	Khalimbekov
1956	Switzerland	Burgisser
1956	Poland	Hauptman et al.
1956	Australia	Irvine
1956	Hungary	Sályi & Hirt
1957	Bulgaria	Ivanov
1958	Shetland	Young & Firehammer

Abortion. Where abortion is caused by L. monocytogenes it usually occurs late in gestation. More often than not the aborting ewe shows no other symptoms except perhaps slight depression. The organism has been isolated from aborted foetuses (Paterson, 1940a; Poppensiek, 1944). Further reports of abortion have been made by Olson (1945), Charles (1950), Csontos & Pesti (1955), Ivanov (1956, 1957), Diplock (1957) and Romváry (1959). L. monocytogenes has been isolated from the genital organs of ewes by Schoop & Gassmann (1943), who described a bacterial venereal disease of sheep due to a mixed infection of Corynebacterium pyogenes and L. monocytogenes. Eide (1956) failed to find L. monocytogenes in the genital organs of normally fertile ewes.

Lambs. Listeriosis may occur as encephalitis (Muth & Morrill, 1942) or as a general septicaemia (Gray, Nelson & Thorp, 1949; Sandvik et al., 1958). Other outbreaks in lambs have been described by Cross (1941); Gray, Johnston & Thorp (1951); and by Watson & Hunter (1958).

Deer. One case has been recorded in Germany by Thamm (1957).

Horses. A few sporadic cases have been recorded in young animals, they may be septicaemic (Grini, 1943; Svenkerud, 1948) or encephalitic (Krage, 1944; Belin, 1946; de Vries & Willems, 1957; and Tabuchi et al., 1951).

Pigs. Pigs with listeriosis usually show little systemic disturbance other than encephalitis, although oedema of the eyelids has been reported as frequently present (Koznov & Barsukov, 1957). Paralysis usually appears first in one or two limbs and progresses until death intervenes in two to three weeks. Occasionally the disease becomes septicaemic and death is rapid. In such cases, multiple necrotic foci are found in the parenchymatous organs.

Encephalitis. The first case was described by Biester & Schwarte (1941a). Further reports are given by de Blieck & Jansen (1942), Helmboldt et al. (1951), Eveleth, Bolin & Turn (1953), Solomkin (1954), Gavrichenkov (1955), Stoyanov (1956), Popov (1957), Koznov & Barsukov (1957), Hauptman (1958), and Ryu (1955).

Non-encephalitic form. Kerlin & Graham (1945) described a case in which the only clinical signs were intermittent diarrhoea, no weight gain and listlessness. In this case L. monocytogenes was isolated from the liver. A similar case was reported by Vendrov (1954) but in addition there was oedema of the leg joints.

Mixed infections. Occasionally L. monocytogenes has been isolated from pigs with swine fever (Zeller & Mayer, 1957; Rhoades & Sutherland, 1948). Pivnik et al. (1958) described a simultaneous infection of L. monocytogenes and an influenza virus. L. monocytogenes has also been found, apparently as a harmless contaminant, in the liver of a pig dying from lye poisoning (Bolin & Eveleth, 1951).

Piglets. In 1938, Slabospits'kiĭ examined swine-pox like lesions in piglets. He isolated a Gram-positive rod and designated it L. suis. The symptoms described are not typical of listeriosis and the identification of the organism is open to question.

Outbreaks of listeriosis have been recorded in Switzerland (Schlegel -Oprecht, 1955); in Russia (Ponomarev, 1957); in the Netherlands (Hessen, 1957; de Blieck & Jansen, 1943).

Rodents. Listeriosis usually occurs in rodents as an extensive septicaemia. Lesions are similar to those already described in lambs.

Rabbits. The first naturally-occurring infection was observed in laboratory animals by Murray et al. (1926) as described previously. There may be severe infection of the female genital organs (Paterson, 1940b; Wramby, 1944; Schoop, 1946), possibly with more extensive occurrence of the organism in the spleen, liver and myocardium (Paterson, 1940b). In pregnant animals infection results in abortion (Jansen, 1945).

Listeriosis may appear as encephalitis with torticollis, impaired balance and difficulty in prehension of food (Traub, 1942; Lesbouyries, 1943).

Chinchillas. Chinchillas appear to be highly susceptible to listeriosis. The signs are usually torticollis and abdominal pain followed by rapid death, but sudden death may occur with apparently no warning signs. On post-mortem examination, foci are found in the liver, spleen, kidney, and brain cortex, and possibly in the mucosa of the intestine (Shalkop, 1950). In addition there may be slobbering, snuffling, opacity of the cornea and haemorrhages in the heart and lungs (Smith, 1953).

Other outbreaks in chinchillas have been recorded by Leader & Holte (1955), Mayer (1956), Jacotot et al. (1956) and Hellmann (1957).

Cases in a number of other rodent species have been recorded and these are tabulated (see Table 2).

Birds. Listeria infection in birds is usually septicaemic with massive necrotic foci in the liver, myocardium and elsewhere. Occasionally the c. n. s. is affected and paralysis or torticollis may result.

Pathology. Gray (1958a) states that there are no pathognomonic signs or lesions in birds with listeriosis. Circulatory monocytosis is not found consistently. Frequently fibrinous pericarditis occurs and the pericardial sac is distended with fluid. Massive areas of myocardial degeneration with engorged peripheral blood vessels may occur, and degeneration rapidly leads to necrosis. Necrotic foci are also found in the liver, spleen, lungs and kidneys. Air sacs may be inflamed and ulceration found in the ileum and caeca. Conjunctivitis occurs occasionally. A few cases show focal necrosis in the c. n. s.

Listeriosis may occur as:

(1) an epidemic in young stock, when discrete necrotic areas are usually found in the liver and spleen, or
(2) as sporadic cases in adults, when massive necrosis of the myocardium is usually found.

In both forms fluid in the body cavity and gelatinous oedema of musculature are almost constant findings.

Fowls. One of the first cases of fowl listeriosis was recorded by Swanstone (1935) and massive myocardial necrosis was found. Typical

Table 2

Reports of listeriosis in rodents other than rabbits and chinchillas

Rodent	Author	Place	Notes
Guinea-pig	Murray et al. (1926)	G.B.	
"	Wramby (1944)	Sweden	
"	Viera & Castelo (1944)	S. America	First case in an animal in Uruguay
Gerbil	Pirie (1927)	S. Africa	
"	" (1938)	"	Epidemic described
Rat	Macchiavello (1942)	Argentina	
"	Pivnik et al. (1958)	Russia	
Lemming	Plummer & Byrne (1950)	Canada	
"	Barrales (1953)	"	
Voles	Levy (1948)	G.B.	
"	Kratokhvil (1953)	Russia	
Hares	Wramby (1944)	Sweden	
"	Henricson (1943)	"	

Carnivora. Few cases have been recorded in carnivores and these are tabulated (Table 3).

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epidemics of listeriosis in young birds have been described by Paterson (1937) and Cole (1941). Sporadic cases in older birds have been described by Bolin & Turn (1951) and Paterson (1937).

Some unusual cases have been found. One was an encephalitis with isolation of L. monocytogenes from the brain only (van der Vlerk, 1954), and in another the liver and kidney showed fibrosis in large areas (Thompson, 1954). Further case reports have come from Germany (Pallaske, 1941), U.S.A. (Hurt et al., 1941; Hoffman & Lenarz, 1942), Italy (Sparapani, 1946), East Prussia (Pothmann, 1944), the Netherlands (Peperkamp & Jansen, 1947a), Poland (Chodkowski & Czarnowski, 1951), Australia

Table 3

Reports of listeriosis in carnivora

Carnivore	Author	Place	Notes
Fox	Cromwell et al. (1939)	Mid-West U.S.A.)	In both cases the disease was at first wrongly diagnosed as distemper
	Peperkamp & Jansen (1947b)	The Netherlands)	
Dog	Chapman (1947)	N. America	Blood counts showed there were 25% monocytes (normal average 5.2%)
Cat	Held (1958)	Germany	
Ferret	Morris & Norman (1950)	U.S.A.	L. monocytogenes isolated from lungs and spleen of apparently healthy ferrets
Raccoon	Gifford & Jungherr (1947)	Connecticut	
"	Osebold et al. (1957)	California)	A decline in numbers of raccoons and skunks was associated with listeriosis in the area and L. monocytogenes was isolated from one skunk
Skunk	"	")	

(Jones, 1953), and Russia (Kornilova, 1956). Mixed infection with L. monocytogenes and Erysipelothrix rhusiopathiae occurring in fowls was described by Juslin & Stenberg (1954) in Norway.

Listeriosis in birds other than fowls. Belding & Mayer (1957) described the first case in turkeys; two forms of the disease were observed. In one there was torticollis, tremors of the skeletal muscle, and depression followed by sudden death; and in the other, white necrotic foci were found on the liver and there was greenish discoloration of the

lungs. The usual visceral lesions noted in other birds were not found. Sporadic cases in geese have occurred in Ceylon (Bandaranayake, 1953) and Hungary (Csontos et al., 1955).

Listeriosis has also been recorded in canaries (Bigland, 1950; van der Schaaf & de Jong, 1951), in capercailzie (Lilleengen, 1942), and L. monocytogenes was isolated from the liver of a blue eagle in Leipzig Zoo by Schultze (1950).

Fish. One case occurring in trout has been recorded by Stamatin et al. (1957).

The aetiology and epidemiology of listeriosis. The sudden occurrence of listeriosis with no apparent source of infection has often proved a puzzle, but on perusal of the literature a number of points become clear. There are three possible reservoirs of L. monocytogenes:

1. Wild animals. The danger to domestic animals will depend on the closeness of contact but pasture contamination may occur. Also infected wild animals may wander aimlessly far from their natural haunts. Examples of occurrence in wild animals are given by Pirie (1927) in gerbils, Plummer & Byrne (1950) in lemming, Osebold et al. (1957) in raccoon, Levy (1948) in voles.

Osebold et al. (1957) carried out an epizootiological study of listeriosis; 107 wild animals and birds were collected in an area where sheep and other animals were infected. Raccoons and skunks showed a decline in numbers during the time when ruminants were affected and L. monocytogenes was recovered from one skunk.

2. Domestic animals acting as symptomless carriers. A number of outbreaks have been attributed to symptomless carriers. In such cases, the disease has occurred, apparently cleared up, and then broken out again after several months. Such a case is recorded by Jungherr (1937). The organism has occasionally been found as a harmless commensal, for example Bolin & Eveleth (1951) recovered L. monocytogenes from a pig, Felsenfeld (1951) from a bird and Morris & Norman (1950) from ferrets.

3. L. monocytogenes may survive outside the host's body. In a case of heavy infection, L. monocytogenes is excreted in the faeces (Osebold et al., 1957). The organism was isolated from faeces in a sheep pen by Sandvik et al. (1958) and has survived at least three months in this habitat. Cases have been recorded of infection occurring in animals which have been put on pastures or in pens previously used by infected animals. Such cases are recorded by Jensen & Mackey (1949) and Pounden et al. (1947). Popov (1957) isolated L. monocytogenes from some waste millers' meal and the organism survived in this for 154 days in the dark at -4°C. to 15°C. Cole (1941) and Lesbouyries (1943) both noted that L. monocytogenes is able to survive in the soil for long periods.

The spread of infection has been shown to occur from animal to animal. The disease may spread in a flock or herd but seldom are all the animals involved. In the U.S.A. the disease seems to occur most severely in "feed-lots" and only occasionally on open range. This is in contrast to European countries where listeriosis occurs more often in animals at

pasture. The infection may pass between different animals, e.g. from sheep to piglets (Hessen, 1957), and from cattle to sheep (Poppensiek, 1944).

Blood-sucking arthropods are potential vectors. Mamedov (1957) reported listeriosis in cattle in Azerbaijan as causing both encephalitis and abortion. He isolated L. monocytogenes from ticks present on the cattle and found that the incidence of disease was related to the extent of tick infestation. Previously Kratokhvil (1953) had found L. monocytogenes in the excreta of ticks.

Spread may occur, too, through milk or meat. It is possible that L. monocytogenes may survive in milk. Dedié & Schulze (1957), working with 54 strains of the organism, found that they were destroyed by heating to 65°C. for 35 sec., 75°C. for 10 sec., and 85°C. for 2 sec. Commercial flash pasteurization at 85°C. killed a mixed culture of the 54 strains but the margin of safety was considered to be low. Bearns & Girard (1958) further found that an initial load of 5,000 to 50,000/ml. was necessary for the organism to survive pasteurization. It was noted that L. monocytogenes added to sterilized milk caused no change in smell or appearance. It should be remembered too that L. monocytogenes is able to grow at refrigeration temperatures (Gray, Stafseth, Thorp, Sholl & Riley, 1948) and could outgrow other organisms and become dominant. L. monocytogenes has also been isolated from mastitis milk.

Naturally-occurring listeriosis has not yet been traced to a contaminated milk supply, although this would constitute a danger if a sufficient number of organisms were present and some were able to survive pasteurization. There would seem to be a very real danger where the milk is not pasteurized.

L. monocytogenes may occur in the meat of an infected animal and the danger constituted has been emphasized by Kampelmacher (1956), v. Stuckrad (1956), Hessen (1958) and Jentzsch (1958). Selivanov & Grinitsuina (1958) found viable organisms in meat even after 20 days' pickling in brine. However Slivko (1958) was unable to recover the organism from the muscle of a naturally-occurring case although it was recovered from the parenchymatous organs and the heart. Again there have been no proven cases of infection from meat; nevertheless it is a potential source of danger.

The uncertainty of the aetiology of listeriosis has led to the implication of many ill-defined predisposing factors such as feed, breeding, weather and general fitness of the animal or bird.

Feed. Listeriosis has been related to silage feeding on a number of occasions (see, e.g. Young & Firehammer, 1958). But Olson, Bagdonas et al. (1953) found that resistance to L. monocytogenes was not reduced by feeding with silage. Jones (1954) thought a case in a nine-month-old heifer might be correlated with a recent change in feed coupled with bedding on mouldy hay. Most recently Hyslop & Osborne (1959) recorded an outbreak in a flock in which the disease seemed to be confined to single lambs whose dams received hay in addition to grazing, clinical infection did not appear in twin lambs whose dams received a supplement of kale.

Breeding. This applies particularly to birds. Fowls show a high natural resistance, but where close inbreeding has been practised, the birds may become susceptible to disease. Jones (1953) suggests inbreeding as a predisposing factor in an outbreak which he studied.

Weather. Bad weather has been suggested as a contributory factor. Vendrov (1954) noted that the disease in pigs was seasonal, being more prevalent in summer than in winter. The reverse had been observed in the U. S. A. (Spencer et al., 1944).

Condition of animal. Pregnant animals seem to be more susceptible to listeriosis as are those in poor condition and infested with internal parasites. Cases have also been reported in pigs following castration (Eveleth et al., 1953), and it was supposed that L. monocytogenes gained entrance through the wounds.

Although listeriosis is still little known, reports are increasing and it may be that, as other more prevalent diseases are brought under control, it may become of greater importance.

Serology. Attempts to protect against listeriosis by the use of serum and vaccines have been largely unsuccessful.

Passive immunity. Osebold & Sawyer (1957) injected mice with immune serum prepared in sheep and rabbits and after 24 hours challenged them with L. monocytogenes, using a series of tenfold graded doses. All succumbed to infection as did the controls. Julianelle (1941a) obtained similar results.

Ryff & Lee (1948) found that immune serum failed to protect sheep even when given simultaneously with L. monocytogenes. However, Olson, Cook & Bagdonas (1951) found one recovered sheep whose whole blood cured three others infected with listeriosis. Blood from another recovered sheep did not have the same effect.

Active immunity — living cultures. Olson et al. (1951) attempted to immunize sheep by giving two subcutaneous injections of living L. monocytogenes with a weekly interval. After the first dose there was only one case in the following week, but after the second dose there were more cases in the vaccinated than in the controls.

Osebold & Sawyer (1955a) reported some success in sheep immunized by repeated subcutaneous inoculation with virulent L. monocytogenes. On intravenous challenge after 25 days none of the immunized sheep developed encephalitis whereas the controls did.

Osebold & Sawyer (1957) failed to establish any immunity in white mice given sub-lethal doses of L. monocytogenes, but Hasenclever & Karakawa (1957) did establish some degree of immunity in white mice by the inoculation of sub-lethal doses. They found that the level of immunity varied with the route of challenge, and that immune animals were most resistant to intra-abdominal and least resistant to intra-cerebral challenge.

Active immunity — dead cultures. Griffin & Robbins (1944b) successfully immunized rabbits with killed cultures. Graham & Levine (1943) attempted to immunize sheep with a culture killed by 0.5% formalin. Injected into three sets of sheep kept in contaminated buildings it gave the following results:

Treatment	% developing disease
(1) one dose 20 ml. — subcutaneous	4.4%
(2) two doses — 20 ml. — " at one week interval	4.9%
(3) three " — 20 ml. — " " " " "	3.4%
(4) Control	6.2%

They considered immunogenic power was feeble.

Description of types. In 1939, Julianelle & Pons working with eleven strains found two types — one isolated from rodents and one from ruminants. In the same year Paterson (1939a, b) found that 27 strains had a common antigen. Other antigens divided the organisms into four groups (Paterson, 1940). He investigated 54 strains and demonstrated both H and O antigens and, besides a common factor B, there were three others — A, C and D in the flagellar system. In the O antigens he distinguished three somatic groups as follows:

Groups with factors I, II & (III)
" " " II, IV & (III)
" " " V & (III)

and thus four types were distinguished as follows:

Type 1 with factors I, II, (III) AB
" 2 " " I, II, (III) DB
" 3 " " II, IV, (III) AB
" 4 " " V, (III) ACB

He regarded the common factor (III) as unimportant and considering the origin of the strains, stated that: "bacteriological types do not bear any relation to the zoological species or geographical distribution of places of isolation".

However, Julianelle (1941a) still maintained that there were only two serological types and Drew (1946) supported this view of one type related to rodents and the other to ruminants.

Seeliger (1954) distinguished two sub-types, 4a and 4b, with a common factor V. Further study by Donker-Voet (1958) revealed three serotypes in Type 4:-

4a (III), V, VII, IX ABC
4b (III), V, VI "
4c (III), V, VII "
4d (III), V, VI, VIII "
4e (III), V, VI, VIII, IX "

and Donker-Voet (1959) differentiated three more groups:-

1b	I,	II,	(III)		ABC
3b	II,	IV,	(III)		ABC
4ab	V,	VI,	VII,	IX, (III)	ABC

All the evidence indicates that there is little correlation between antigen, species, and area.

Antibody production. The level of antibodies formed as a result of the introduction of L. monocytogenes into the blood stream is variable. Janeway & Dammin (1941) found no rise or fall of agglutinins during the course of listeriosis although the controls had slightly lower titres.

The variability of antibody production is illustrated by Hoffman & Lenarz's work (1942). They inoculated chickens and rabbits with strains isolated from chickens and sheep. Of the sera produced, complete agglutination was given by 21 in a dilution of 1/50; 20 in a dilution of 1/100; 12 in a dilution of 1/200 and 4 in a dilution of 1/800. Seeliger (1953) considered that H & O agglutinins in patients' serum were diagnostic if the titre was 1:320 or more. Later (1956) he considered that a titre of 1:10 to 1:320 indicated active recent infection and that 1:640 indicated a definite infected state. But Seeliger states that isolation of the organism is the only certain diagnosis. This latter point is emphasized by Osebold & Sawyer (1955b) who found that antibodies were present in healthy people. The sera of 468 people were studied and they suggest that the antibodies present may have been the result of inapparent infection, or the result of some other antigen.

Robbins & Griffin (1944) studied the effects of the method of killing the organism on antibody production. They found that minimum heat, formalin, chloramine-T, thiomersal and phenol had little effect. Flagellation factors B and D were not affected, though A was degraded by the last three and C by phenol. In 1945, Robbins & Griffin further found that heat and alcohol did not affect the capacity of the organism to stimulate antibody production. Griffin & Robbins (1944b) stress the need for a constant temperature to keep the flagellar picture constant.

Change in blood picture. Olson, Cook & Blore (1950) found that after injection of L. monocytogenes neutrophiles increased and lymphocytes decreased. Immature neutrophiles increased as there was a sudden and rapid demand for these cells. Sheep previously exposed are more resistant to subsequent exposure and show less deviation in blood picture.

Antibiotics and chemotherapy. Porter & Hale (1939) showed that infected mice were effectively protected if dosed with sulphanilamide or sulphapyridine early in the infection. In the controls there were 70/80 deaths, whereas with sulphanilamide treatment there were only 4/60 deaths, and with sulphapyridine 2/20. Savino (1940a) found that sulphapyridine protected rats.

Biester & Schwarte (1941a) suggested that a single large dose of sulphanilamide might influence the course of infection in pigs in field

outbreaks. But Henderson (1941), Jensen & Gay (1941), Graham, Levine & Morrill (1943) reported sulphanilamide as being ineffective in the treatment of cattle and sheep.

In rabbits, however, Ryff & Lee (1948) found sulphapyridine gave the most promising results in experimental therapy. Schoop (1946) controlled an outbreak of infectious metritis in Angora rabbits using "Prontosil", but recovered does were sterile.

Shalkop (1950) found that administration of a triple sulphonamide (containing sulphathiazole, sulphadiazine, and sulphamerazine) halted deaths occurring in a group of chinchillas. But he considered it might have been the natural termination of the disease. Hessen (1957) reported successful treatment of piglets with sulphadimidine administered intraperitoneally.

Table 4

Table to illustrate different minimum inhibitory concentrations found by various authors

		Antibiotics (penicillin in units/ml.; others in μg./ml.)				
Author	Year	P	S	CT	CA	O
Beute et al.	1948	0.63	1.25			
Stanley	1948	0.31	6.25			
Norman et al.	1951			0.05	0.83	
Zink et al.	1951	16.6	160.0	0.04	20.0	2.5
Bennett et al.	1952	0.5	1.25	0.75	3.15	0.37
Felsenfeld et al.	1950			0.05	0.83	

CT=Chlortetracycline O=Oxytetracycline P=Penicillin
S=Streptomycin CA=Chloramphenicol

Savino (1940b) reported a case of listeriosis in man cured by oral and intramuscular administration of sulphapyridine. However, Stanley (1948) found a strain isolated from a man which was relatively insensitive to sulphapyridine, sulphadiazine, and sulphanilamide.

Antibiotics in control of outbreaks and in in vitro tests have given widely conflicting results. Some of these are indicated (Table 4) and the order of effectiveness found by various authors (Table 5).

Penicillin. The ineffectiveness of penicillin was first noted by Foley et al. (1944), who found that seven strains grew freely in forty

times the concentration of penicillin normally sufficient to inhibit completely the growth of other representative Gram-positive organisms. Stanley (1948), Ortel (1955) and Dedrick (1957) also found L. monocytogenes to be insensitive to penicillin. On the other hand, penicillin gave the most promising results in therapy of: rabbits (Ryff & Lee, 1948), sheep (Olson, Cook & Bagdonas, 1951) and pigs (Vendrov, 1954).

Chlortetracycline and oxytetracycline.

Chlortetracycline (Aureomycin) has been found to be effective in the treatment of encephalitis in rabbits (Gray, Laine & Thorpe, 1952) white mice (Ortel, 1955), infections in cattle but not in sheep (Gray & Moore. 1953). In vitro tests also showed the organism to be sensitive tc Aureomycin (Zink et al., 1951; Dedrick, 1957). However, Roine et al.

Table 5

Table to show order of effectiveness found by various authors

Author	Year	Decreasing effectiveness →				
Özgen	1952	O	P	CT	CA	S
Sandvik et al.	1958	S	CT) O)	P		
Ortel	1955	CT		P) S) CA)		
Dedrick	1957	CT) O)	CA	P		

CT=Chlortetracycline O=Oxytetracycline P=Penicillin
S=Streptomycin CA–Chloramphenicol

(1955), working on the effect of antibiotics on the intestinal flora of guinea-pigs, found that dosage with Aureomycin allowed an increase in L. monocytogenes so that the animals died from the infection. This only occurred on oral administration of the antibiotic. The same effect was observed if Aureomycin and Terramycin were administered together orally.

Again there are conflicting reports concerning oxytetracycline (Terramycin). Zink et al. (1951) found the organism to be insensitive and Ortel (1955) sensitive.

Chloramphenicol. Zink et al. (1951) and Ortel (1955) found chloramphenicol to be ineffective but Dedrick (1957) found it to be effective in inhibiting L. monocytogenes.

Streptomycin. Streptomycin is recorded as having little

effect by Özgen (1952), Ortel (1955), and Stanley (1948). Sandvik et al. (1958), however, found streptomycin to be effective in vitro. These conflicting results may be explained by the work of Gray, Stafseth & Thorp (1949), who found that streptomycin was inhibitory at first but that rapid adaptation to this antibiotic occurred.

Other antibiotics. The organism is sensitive to aspergillin (Stanley, 1948); to neomycin and to polymyxin D (Felsenfeld et al., 1950) and slightly sensitive to thermomycin (Schöne, 1951).

Experimental animals. Animals have been used experimentally in (1) the characterization and diagnosis of the disease, and (2) in investigating the aetiology of the disease and modes of transmission.

Results of the experiments falling into the first group have been tabulated (Table 6).

Table 6

Listeriosis in experimental animals

Animal	Route	Manifestation	Author
Pregnant heifer	Intravenous	Abortion. L. monocytogenes recovered from foetus only.	Graham, Hester & Levine (1940a)
Steer	Rubbed on conjunctiva	Conjunctivitis (slight)	Graham, Hester & Levine (1940b)
Heifer	Intracerebral	Death	Graham, Hester & Levine (1940b)
Sheep	"	"	"
Sheep	Intravenous	Ill, but recovered	"
Sheep	Rubbed on conjunctiva	Conjunctivitis	"
Lamb	Intravenous	c.n.s. symptoms	"
Horse	Intracerebral	Death	"
Horse	Intravenous	Ill, but recovered	"
Horse	Rubbed on conjunctiva	Conjunctivitis	"

Animal	Route	Manifestation	Author
Swine	Intravenous	Incoordination, trembling, stilted gait of forelegs. Perivascular cuffing.	Biester & Schwarte (1940)
Pregnant swine	"	Death in a few days.	Graham, Hester & Levine (1940a)
Pig	"	Ill, but recovered.	Graham, Hester & Levine (1940b)
Pig	Rubbed on conjunctiva	Death	"
Pig	Intravenous	"	de Blieck & Jansen (1945)
Rabbit	"	Tubercles on lungs. Necrosis of liver.	Webb (1935)
Rabbit	Rubbed on conjunctiva	Conjunctivitis localized in eye.	Julianelle & Pons (1939) Julianelle (1941a, b) Julianelle & Moore (1942)
Rabbit	Intravenous	Circulatory monocytosis.	Paterson (1939a)
Pregnant rabbit	"	Death in a few days.	Graham, Hester & Levine (1940a)
Rabbit	Rubbed on conjunctiva	Conjunctivitis	"
Rabbit	Intravenous	Death	Ryff & Lee (1948)
Rabbit	Intracerebral	Paralysis of hind legs and sphincter muscles. Death in 14 days. Organism isolated from brain and liver.	Zink et al. (1951)
Rabbit	Subcutaneous	No clinical symptoms, liver foci, fibrous pericarditis, myocarditis.	"

Animal	Route	Manifestation	Author
Rabbit	Subcutaneous	Conjunctivitis, liver foci, slight myocarditis, though apparently healthy.	Zink et al. (1951)
Rabbit	Intravenous	Death. Necrosis at point of inoculation.	Lilleengen (1942)
Rabbit	"	Death.	de Blieck & Jansen (1943)
Pregnant rabbit	"	Abortion.	Jansen & van der Hurk (1945)
Rabbit	Organism in a collodion capsule placed in the subcutaneous tissue of the abdomen.	In non-pregnant rabbit, local inflammation only was seen. In pregnant rabbit—organism isolated from placenta, uterus and foetuses. Lesions not always produced. Clinical manifestation only when there were lesions in uterus.	Suchanová et al. (1958)
Pregnant guinea-pig	Intravenous	Death in a few days.	Graham, Hester & Levine (1940a)
Guinea-pig	Rubbed on conjunctiva	Conjunctivitis	"
Guinea-pig	Subcutaneous	Conjunctivitis in 48 hrs. Keratitis in 3-4 days.	Zink et al. (1951)
Guinea-pig	Intravenous	Death. Necrosis at point of inoculation.	Lilleengen (1942)
Guinea-pig	"	Death	de Blieck & Jansen (1943)
Rat	"	"	"

Animal	Route	Manifestation	Author
Pregnant rat	Placenta at third day of pregnancy	a) death pre-term b) abortion c) slight brown discharge at end of pregnancy. Post-mortem maternal organs normal, foetuses dead and being resorbed, some moribund, some normal. L. monocytogenes recovered from uterus and placenta.	Payne (1958)
Mouse	Intravenous	Increase in monocytes to 5.1% in 5 days. Increase in granulocytes to 7.0%.	Stenberg (1955)
Mouse	"	Death. Necrosis at point of inoculation.	Lilleengen (1942)
Mouse	"	Death.	de Blieck & Jansen (1943)
Hamster	Intracerebral	Death in 24 hrs. Organism recovered from brain, liver and cornea.	Zink et al. (1951)
Dog	Intravenous	Slight indisposition.	Graham, Hester & Levine (1940b)
Puppy	Rubbed on conjunctiva	No result.	"
Cat	Intracerebral	Ill, but recovered.	"
Cat	Intravenous	"	"
Chicken	"	Necrosis of myocardium	Graham, Hester & Levine (1940a)
Chicken	Intracerebral	Death.	Graham, Hester & Levine (1940b)

Animal	Route	Manifestation	Author
Chicken	Subcutaneous	No reaction.	Graham, Hester & Levine (1940b)
Chick	Intra-peritoneal	Monocytosis.	Cole (1941)
Chicken	"	Negative.	Zink et al. (1951)
Fowl	Intravenous	No effect.	Lilleengen (1942)
Chick embryo	Rubbed on conjunctiva	Similar to virus lesions on the chorioallantoic membranes, liver heart and c.n.s.	Paterson (1940c)
Turkey 3–11 days	Intravenous	In 16–20 hrs. drooping head, nervous symptoms, hunched, paralysis. 24–80 hrs. death. Haemorrhagic epicardium. Isolates from the heart, liver and brain of 4/5.	Malewitz et al. (1957)
Pigeon	"	Death. Necrosis at point of inoculation.	Lilleengen (1942)
Canary	"	Death.	de Blieck & Jansen (1943)
Capercailzie	"	Death. Necrosis at point of inoculation.	Lilleengen (1942)

Pathogenesis and transmission. Olson & Segre have done some work towards finding out if there is some additional aetiological factor involved in listeriosis besides the organism itself and, in 1956, they isolated a "listeriosis-enhancing agent" from the defibrinated blood of sheep. But it could only be demonstrated by simultaneous injection with the organism. In 1957, Olson, Grace et al. reported that blood, lymph node extract and saliva from a case of mucosal disease mixed with L. monocytogenes enhanced the development of listeriosis in exposed sheep. This led to the idea that viruses might be capable of potentiating bovine and ovine listeriosis.

Segre et al. (1956) used anti-metabolites which interrupted the

tri-carboxylic acid cycle to see if the course of listeriosis in chickens was interrupted, but the results were negative.

Gray, Singh & Thorp (1955) carried out a series of experiments on the effect of the route of inoculation. Ocular instillation into pregnant rabbits resulted in abortion, if gestation was in the early stages, and in still-birth or early death of the young if gestation was in a later stage. Non-pregnant controls showed no symptoms--although there may have been a transient infection—but high agglutination titres were found. Does re-bred one week after delivery of a litter, which died of listeriosis, aborted spontaneously in the third week of gestation; however, re-breeding after 17 days resulted in a normal litter. Oral administration in drinking-water to pregnant rabbits gave similar results. A similar administration also proved fatal to young ewes and goats (Gray, Singh & Thorp, 1956). In animals early in gestation abortion was caused, late in gestation—death occurred in seven days. Two dams died from listeria septicaemia. But Gray (1958) further noted that if infected foetuses, of rabbits, sheep, or goats, were rapidly expelled then no symptoms were seen in the dam.

Gray (1958) found that conjunctival instillation in rabbits had a similar effect to oral administration. Vaginal instillation just before parturition resulted in death of some of the young, c.n.s. disorders in others, and had no effect on the rest. Asahi, Hosoda & Akiyama (1957) in further work on conjunctival instillation found lesions similar to those occurring naturally on the trigeminal nerve leading from the eye to the brain stem. Similar lesions were found in rabbits after application through minute oral and nasal mucous membrane wounds, or by feeding boiled dried fish soaked in L. monocytogenes. A route of invasion of the nervous system is thus suggested.

The organism has been shown to be transmitted through the dam's milk. Gray & Thorp (1957) exposed female rabbits to oral infection either just before or just after parturition and 7/11 litters were found to contain one or more infected offspring. Sixteen new-born rabbits, whose dams were uninfected, all died within 7 days after being suckled by infected does.

Khalimbekov (1957) established the role of symptomless carriers. Ten clinically recovered animals were placed in a flock of two hundred infection-free sheep and goats and the flock was isolated. After 70 days deaths due to listeriosis began to occur. During August to October, 12 animals were affected, and from June to October in the following year 18 animals were affected. The organism was isolated on occasions from nasal and ocular discharges, urine, milk, genital organs, and semen.

Isolation from infected tissue. The isolation of L. monocytogenes from tissue has proved difficult with many failures despite all the symptoms pointing to the presence of the organism. Gray, Thorp et al. (1947) isolated the organism from ewes and lambs by shaking infected brain tissue with tryptose broth and then plating out on blood agar. The growth obtained was injected into rabbits, and the organism recovered from brain and liver. However, this proved laborious and it was found (Gray, 1948) that refrigeration of suspect tissue for 5–13 weeks markedly increased the number of isolates obtained. Gray postulates that this may be due to:-

(1) the fact that growth occurs readily at 4°C., whereas most organisms do not grow at 4°, and so L. monocytogenes would become dominant, or
(2) disintegration of the tissue may release more organisms, or
(3) that an unstable inhibitory factor may be present which is destroyed during refrigeration.

General comment. It will be realized from the preceding detailed account of the manifestations and approaches to the therapy of listeriosis in animals that infections due to Listeria monocytogenes are common throughout the world.

Very recently, two further papers have appeared which underline the scientific interest in the organism (see, e.g. Hyslop & Osborne, 1959) and its ranking possibly as one of the zoonoses. Smith et al. (1960) have been using the fluorescent antibody technique as a means of identifying the organism and describe the preparation of a polyvalent somatic fluorescein-labelled antiserum specific for 30 strains from human and animal sources. Rappaport et al. (1960) have demonstrated a relationship between the organism and repeated abortion by the isolation of L. monocytogenes from the genital organs of 25 out of 54 women with a history of repeated abortion. The organisms were also isolated from aborted products of conception. Treatment with penicillin and sulphamethoxypyridazine gave satisfactory results.

It is likely, therefore, that further studies with this organism will be forthcoming, the results of which, it is hoped, will be of value both to man and his animals.

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Vibrio fetus Infection in Sheep

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INTRODUCTION

Vibrionic abortion in sheep was first recognized in England by M'Fadyean and Stockman in 1909. Their report on abortion in sheep, published in 1913, described the disease and drew attention to the importance of vibrionic abortion in ewes. They recorded that 14.7% of 476 farms experienced an abortion rate of 10% or more, during a five year period. The abortion rate varied from 5–50%.

Vibrionic abortion has since been reported in the U.S.A. (Carpenter, 1920; Welch & Marsh, 1924; Graham & Thorp, 1930; Gilman, 1934; Baker & Stone, 1938; Ryff, 1940. Lee & Scrivner, 1938, 1941; Prier, 1952, Biswal et al., 1953; Wiggins, 1955): in Germany (Knuth, 1925; Spiegl, 1925; Grimm, 1927; Knuth & David, 1928; Zwick, 1930; Berge & Schoop, 1933; and Miessner & Köser, 1934); in Hungary (Schmiedhoffer, 1926; Marcis, 1934). in Sweden (Klarin, 1926 and Olson, 1946): in France (Vinzent & Alloy, 1952) in Great Britain again (Buxton, 1931; Bosworth & Glover, 1951. and Blakemore & Gledhill, 1946); in New Zealand (McFarlane et al., 1952) in Norway (Hoff, 1953; Hoff & Kaldahl, 1954; Eide & Helle, 1957); in Australia in 1938 (Bain, 1960) in Spain (Blanco Loizelier et al., 1955) and in Poland (Czarnowski, 1956).

Although vibriosis of sheep has been known for fifty years, it has received less attention than bovine genital vibriosis: precise information regarding its prevalence and distribution is not available but it has been described from most of the important sheep breeding areas of the world, and like bovine genital vibriosis is capable of causing serious economic loss.

The organism is believed to be fairly widely distributed among sheep in England and Wales and has recently been recorded in Scotland (Stamp, 1950; Anon., 1955). In Norway the condition appears to be common (Eide & Helle, 1957). Marsh (1956) stated that the only known important cause of infectious abortion in the U.S.A. was Vibrio fetus. Since then ovine virus abortion has been recorded (Young et al., 1958), but information regarding its extent and distribution is not known. Vibrionic abortion in sheep is now recognized in many States of America. Montana has experienced losses since the early 1920's, but reliable statistics are

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not available. The U. S. Dept. of Agric. (Anon., 1954) estimated that vibriosis accounted for more than 70% of the annual loss due to sheep diseases. This is a striking figure in the light of the position in other countries.

For a number of years the disease has been investigated at several experimental stations in the U.S.A., particularly in Montana and Wyoming. Since 1952, when very heavy losses occurred in the Rocky Mountain States (estimated two million dollar loss) intensive research has been carried out in California, Colorado, Idaho, Montana, Utah, Washington and Wyoming on a co-ordinated western regional research project.

Morphological, cultural and biochemical characteristics

The morphological, cultural and biochemical characteristics of ovine isolates are indistinguishable from those of bovine strains of V. fetus (Lawson, 1959) but they appear to grow slightly better than those from cattle (DiLiello et al., 1959). Reich et al. (1956) found ovine strains more strictly anaerobic than bovine strains. Pathogenicity of ovine strains appears to be limited primarily to the foetus, uterus and to the pregnant placenta. Ovine strains were shown to be abortifacient to pregnant guinea-pigs by M'Fadyean & Stockman (1913) and this has been confirmed by numerous workers. Pathogenic are distinguished from non-pathogenic isolates by the criteria used to differentiate cultures of bovine origin.

Identification of ovine Vibrio isolates

Miller et al. (1958) state that two species of Vibrio may be found in the genital organs of sheep. V. fetus is consistently present in aborted foetuses, foetal membranes and uterine exudates from aborting ewes. Also, that V. fetus has rarely, if ever, been found as a natural infection in the genital organs of rams or in the genital organs of ewes after cessation of exudation following abortion. Eide & Helle (1957a, b) reported the isolation of vibrios from animals showing no clinical signs of infection in an experimental sheep flock in which a spontaneous outbreak of vibriosis had occurred. The vibrios were isolated from (1) vaginal mucus from pregnant ewes that later produced normal lambs, (2) vaginal mucus from virgin gimmers (yearlings); (3) foetal fluids from normally lambing ewes (4) stomach contents from normal lambs at birth; and (5) semen from the rams. All these strains reacted similarly to the biochemical tests, were abortifacient in pregnant guinea-pigs and showed serological relationship by cross-agglutination tests. From this flock, 18 months after the occurrence of vibrionic abortion, Eide & Helle (1958) isolated vibrios from vaginal mucus, foetal fluid and foetal stomach contents taken at normal parturition. These vibrios were serologically related to the original isolates, only two strains showed catalase activity, hydrogen sulphide was not produced, slight growth was visible along the stab in the stab cultures and pathogenicity was reduced for pregnant guinea-pigs (35% abortion incidence compared with 72% originally).

The second species of Vibrio (Miller et al., 1958) officially unnamed but for which the name of Vibrio dubius was recommended by a special

subcommittee of the Western Regional Research Project W-27, has been isolated from the prepuce of rams and from the vagina of ewes (Binns, 1954; Miller, unpublished; Firehammer, 1955; Firehammer et al., 1956, and McGowan, 1958). This organism corresponds closely to the non-pathogenic vibrio of cattle as described by Bryner & Frank (1955) in being catalase negative and hydrogen sulphide positive. Although its pathogenicity for sheep has not been studied exhaustively, available evidence indicates that it is non-pathogenic.

Bryans et al. (1960) described outbreaks of vibrionic abortion in two flocks in Kentucky caused by a catalase positive and hydrogen sulphide positive Vibrio sp. There was no relationship between the two flocks which were separated by ninety miles. These isolates were biochemically and antigenically distinct from previously described ovine V. fetus strains. These authors question the reliability of biochemical tests for determining pathogenicity of V. fetus isolates. They consider that the ultimate criterion for designation of a Vibrio isolate as V. fetus must remain the source of the culture. Russell (1955), in New Zealand, reported an unidentified microaerophilic vibrio associated with scouring and mortality in hoggets. He considered it to be possibly related to V. fetus because of its morphological and cultural characteristics.

It is evident that the present methods of classification of ovine vibrios are unsatisfactory.

Serological types

The possibility that several serological types occur in sheep was suggested by Blakemore & Gledhill (1946) and Levi (1950). Cross-agglutination and absorption tests on ovine isolates have confirmed this opinion.

Marsh & Firehammer (1953) reported cross-agglutination tests, using 23 ovine and 3 bovine strains. They classified the ovine strains into 4 types, the majority falling into one type which was sharply differentiated from the other three. The bovine strains constituted a fifth type, which showed relationship to two of the less common ovine types.

Kiggins (1954) divided six ovine strains into three serological types. Price et al. (1955) classified 14 bovine strains and one ovine, finding that the ovine strain was unrelated to the four bovine types. Biberstein (1956) worked with 47 strains, including three ovine strains. He classified two of the ovine strains as a distinct type while the third was similar to the bovine strains.

The results in general indicate that the ovine strains tend to be more heterologous than the bovine and that at least four serological types occur.

Relationship between ovine and bovine vibriosis

The principal bovine type appears to be serologically different from types found in sheep; however, some bovine and ovine strains are closely related or identical.

There appears to be little relationship between outbreaks of ovine and bovine vibriosis. The work of M'Fadyean & Stockman (1913) and Hoff (1953) suggests that cattle can be infected experimentally with ovine

strains. Bu et al. (1955), in an experiment on the pathogenicity of bovine V. fetus strains for ewes, concluded that the risk of transmission of vibriosis from cattle to sheep under practical conditions was small. Eide & Helle (1957) reported that fertility in a cattle herd that had been in contact with a vibrio infected sheep flock, had been normal. Vaginal mucus samples from all the cows were examined for V. fetus with negative results and all the agglutination tests with antigens from the sheep strains against V. fetus positive bovine vaginal mucus samples were negative. Jensen (1958) failed to infect six pregnant cows orally using V. fetus infected sheep tissues. Jensen et al. (1957) challenged 20 ewes in advanced pregnancy orally with bovine foetal tissue infected with V. fetus and produced one abortion.

Cornell workers inoculated eight heifers intra-uterinely with cultures of two strains of catalase positive ovine isolates from aborted lambs. Catalase positive vibrios were isolated from vaginal mucus up to approximately four weeks following inoculation (Wagner et al., 1959).

An interesting note by Lawson (1959) reported that samples of vaginal mucus from ewes which had aborted in a flock infected with vibriosis, gave marked agglutination when tested against the standard bovine V. fetus antigen in a routine mucus agglutination test at Weybridge.

Jensen (1958) considers it important to determine the cross-infectivity between sheep and cattle. If cross-infection is possible, one species may act as a reservoir of infection for the other. Vandeplassche (1959) considers that sporadic V. fetus abortion in cattle may have some connexion with vibrionic abortion in sheep. The observations presented above indicate that this is apparently not the case.

PATHOGENESIS

Vibriosis of sheep, an acute infectious disease, is characterized by abortion in the later stages of pregnancy and is caused by V. fetus. It differs from bovine genital vibriosis in several aspects, namely the route of infection, clinical signs and course of infection.

Mode of transmission

In cattle transmission is venereal and the main symptom, temporary infertility. In contrast, the oral route seems to be the main mode of transmission in sheep and the characteristic symptom is late abortion. Unlike bovine vibriosis this abortifacient infection of ewes is associated with little or no infertility (Hoff & Kaldahl, 1954; Firehammer et al., 1956).

Source of infection. The epidemiology of ovine vibriosis is poorly understood. The usual history is that an outbreak occurs suddenly with no record of exposure to the disease. The available evidence indicates that the disease is self-limiting and the infected ewes lose the infection rapidly and do not act as carriers (M'Fadyean & Stockman, 1913; Baker & Stone, 1939; Ryff, 1941; Biswal et al., 1953; Tunnicliff & Marsh, 1954, Marsh et al., 1954; Wiggins, 1955; and Jensen et al., 1957).

Marsh (1958) states, however, that further work was being done, as it is possible that an occasional individual may serve as a reservoir of

infection. The New Zealand Dept. of Agric. report for 1956–1957 suggested that it may not always be self-limiting, and that in an outbreak in a Southdown stud, the ewes were found to have a residual vibrio infection.

Eide & Helle (1958) reported the persistence of genital vibrio infection 18 months after an outbreak of abortion. They isolated vibrios from vaginal mucus of ewes, virgin yearlings and lambs aged six months. This investigation shows that sheep may carry vibrios long after abortions cease, even when there are no clinical signs of infection in the flock, i.e., "carrier state". These authors suggest that flocks like the one investigated may be the source of infection to susceptible flocks.

Robertson (1959) reported from Scotland the association of V. fetus with acute liver fluke infestation in lambs. V. fetus was recovered from the lumen of the thickened portal tracts together with the liver flukes. The liver of one of the five infected lambs showed numerous greyish white soft raised areas, 1 to 1.5 cm. in diameter. Robertson was unable to trace the source of infection but the report well illustrates our lack of epidemiological knowledge of ovine vibriosis.

Relationship of susceptibility to infection and stage of pregnancy. Ryff & Lee (1945) failed to produce abortion in 2 two-months pregnant ewes given cultures intravenously or orally. Lindenstruth et al. (1949) studied the relationship of susceptibility of pregnant ewes to the period of pregnancy. Groups of four pregnant ewes were challenged orally with hay and water contaminated with V. fetus during each month of gestation and a second series was inoculated intraperitoneally with V. fetus cultures. Vibriosis was established in groups infected orally in the fourth and fifth months and in the third, fourth and fifth month in the intraperitoneally inoculated ewes. Firehammer et al. (1956) found it difficult to infect ewes by any route until the second month of pregnancy. Tucker & Robertstad (1956) fed chopped hay, heavily impregnated with viable V. fetus, to 38 ewes in three groups. Group 1, 75–78 days pregnant, were fed contaminated feed for 11 days, group 2 for 3 days when 92–97 days pregnant; and group 3 for 2 days when 124–127 days pregnant. Abortions occurred 2–3 weeks later in groups 2 and 3. Frank et al. (1957) caused abortion in 11 of 12 ewes infected orally during the last half of the third month of pregnancy. They found ewes to be susceptible in the third, fourth and fifth months. Jensen et al. (1957) inoculated 23 ewes orally with 5 g. of V. fetus infected lamb tissues at the beginning of the fifth month and 73.9% aborted. In a second experiment 241 ewes were divided into nine groups and infected orally. The group, fed infected foetal tissue, had a significantly higher rate of abortion than did the groups fed with either cultures of V. fetus, bacteria-free infected tissue filtrate, manure from an infected pen or bovine foetal tissue infected with V. fetus.

Exposure of susceptible ewes to pathogenic strains of V. fetus at any time after 90 days of gestation may be expected to produce abortion (Tucker & Robertstad, 1956).

Experimental transmission of infection

(a) Oral exposure: M'Fadyean & Stockman (1913) produced abortion by inoculating ewes orally with infected lamb tissue or V. fetus cultures. Oral dosing has since been shown by many workers to result in infection

and abortion (Lee & Scrivner, 1941. Lindenstruth et al., 1949; Tucker & Robertstad, 1956; Frank et al., 1957; and Jensen et al., 1957).

(b) Intravenous and intraperitoneal exposure: This has been shown to be the most reliable method for infecting ewes experimentally. Intravenous inoculation was employed by M'Fadyean & Stockman (1913) and parenteral infection has been recorded by Welch & Marsh (1924), Lee & Scrivner (1941), Slavin & Weddell (1944), Lindenstruth et al. (1949) and Tunnicliff & Marsh (1954).

(c) Conjunctival Sac exposure: Introduction of V. fetus into the conjunctival sac of a pregnant ewe by Lee & Scrivner (1941) and Ryff & Lee (1945) failed to produce abortion.

(d) Vaginal and Os Uteri exposure: M'Fadyean & Stockman (1913) reported negative results with the inoculation of V. fetus culture into the vagina of two ewes that were about two months pregnant. Marsh & Tunnicliff (1955) inoculated 36 ewes at the time of mating by depositing cultures of V. fetus at the os uteri either immediately before or immediately after service. Only four of the 36 ewes developed agglutinins and none aborted.

The interval between infection and abortion observed in all experimental transmission studies was remarkably constant and varied from 7 to 25 days with a mean of 15.2 ± 4.42 (Jensen et al., (1957).

Role of the ram. In contrast to bovine vibriosis the male plays no important part in the transmission of ovine vibriosis. Buswal et al. (1953) failed to isolate V. fetus from the semen of three rams from an infected flock. Firehammer et al. (1956) were unable to infect ewes inseminated with semen to which V. fetus had been added. Rams that were inoculated with V. fetus intravenously or via the preputial cavity, failed to infect ewes, all of which lambed normally. Ewes inoculated with V. fetus either intravenously or into the uterine os, before or at time of mating, failed to become infected. Jensen et al. (1957) were unable to transmit infection by using rams that had V. fetus in their preputial secretions from natural exposure as well as from experimental intrapreputial inoculation.

Since ewes are apparently resistant to infection until at least 60 days pregnant the ram is unlikely to spread infection.

There are only two reports suggesting that the ram may be implicated in transmission. Buxton (1951) in England reported one ram that appeared responsible for vibrionic infection in two small flocks. The ram died a year later and V. fetus was recovered from his heart blood and liver. In Norway, (Eide & Helle, 1957), infection was spread by coitus in the largest section of an infected flock (Breed No. 1) and V. fetus was recovered from the semen of all the rams. These authors isolated the organism from the semen. They gave no details as to the site of infection in the ram; whether it was situated superficially in the prepuce or deeper in the other genital organs.

Natural exposure: M'Fadyean & Stockman (1913) believed the oral route to be the most likely means of natural transmission. Current opinion agrees that oral transmission is the principal method of spread of ovine vibriosis. All the evidence of field and experimental transmission, of susceptibility to infection, and of the isolation of V. fetus from virgin yearlings and 6-month-old lambs, points to oral infection occurring from infected pastures, water, premises, etc. Tucker & Robertstad (1956)

observed that when one ewe in a flock aborts, irrespective of the source of infection, the susceptible animals in the whole flock are endangered. M'Fadyean & Stockman (1913) stressed that ewes were found passing vibrios via the vagina some days before aborting. They quoted a range of 9 to 68 days. Since outbreaks of vibrionic abortion generally occur during the colder months of the year, the work of Lindenstruth & Ward (1948) is of importance. They found that the viability of V. fetus on hay, soil and manure was approximately doubled when the temperature decreased from 20° to 6°C.

The report of Eide & Helle (1958) that animals in the flock may carry vibrios long after abortions cease, even when there are no clinical signs in the flock, is of epidemiological importance. They showed that the sporadic spontaneous nature of the disease may be due to carrier animals. No other natural reservoirs of infection are known.

Course of infection

Little is known of the pathogenesis of V. fetus infection in sheep. Miller et al. (1959) demonstrated bacteraemia in ewes inoculated orally with infected lamb tissues or V. fetus culture. The greatest incidence of bacteraemia occurred during the third day following inoculation, V. fetus was not found in any blood sample after the fourteenth day.

The organisms invade the pregnant uterus, proliferate in the placental tissue resulting in a placentitis, and cause early expulsion of the foetus. The lamb may die in utero, become mummified and be expelled later; or die and become invaded by putrefactive bacteria; or be born dead; or be born alive and weak at full term and die within a few days (M'Fadyean & Stockman, 1913; Anon., 1955; Miller, 1958).

Jensen et al. (1961) have recently suggested the following pathogenesis for ovine vibriosis. A maternal bacteraemia occurs leading to localization of V. fetus in the "hilar zone" of the placentome (vide infra). Many endometrial septa develop arteriolitis and some thrombosis. V. fetus localizes in the haematoma of the lacunae, proliferates and invades and parasitizes epithelial cells and the endothelial cells of the capillaries of the chorionic villi. This results in the vessel lumen being partly or completely occluded by colonies of V. fetus and thrombosis. From these vessels, the organisms have free access to the foetal circulation and foetal liver. The foetus dies because of bacteraemia and possibly toxaemia. Following foetal death, the infected foetus and membranes decompose and autolyse rapidly. The uterine contents, infected with V. fetus, are expelled and contaminate the environment.

The precise mechanism causing abortion is not known. Lipopolysaccharide extracted from V. fetus caused death in utero and abortion when administered intravenously to a small number of ewes in the later stages of pregnancy. The foetal and placental changes were similar to those described for the whole organism (Dennis, 1960). The rapid proliferation of V. fetus within the uterus could lead to a build up of endotoxin which, when it reached a certain threshold, could result in expulsion of the uterine contents. Endotoxins from Gram-negative bacteria are known abortifacients and are considered to be vascular poisons (Thomas, 1954).

That vibrios were found at normal parturition and in the vaginal mucus from pregnant ewes, which later lambed normally, indicates that not every ewe infected with V. fetus shows clinical signs (Eide & Helle, 1957). In addition, tneir finding of vibrios in the vagina of virgin yearlings, suggests that the pregnant uterus is not the only tissue in which vibrios can become established.

Clinical signs of infection

The characteristic symptom of V. fetus infection in sheep is abortion in the later stages of pregnancy. The usual history is, that a few ewes aborted in the early stages of the breeding season, and the abortion "storm' appeared about six weeks before the flock was due to lamb (Stockman, 1919; Tucker & Robertstad, 1956; and Marsh, 1956). The abortion rate varies from 5 to 50%, may be as high as 70% and usually between 10 to 20% (M'Fadyean & Stockman, 1915; Marsh, 1956; Jensen et al., 1957), but Czarnowski (1956) and Blakemore & Gledhill (1946) encountered outbreaks with abortion rates of 80–90%. A mortality rate of approximately 5% occurs in aborting ewes as a result of uterine sepsis. There are no well defined clinical signs preceding abortion, but close observation may reveal a vaginal discharge several days before abortion and the ewe may appear sick. The ewes usually have a brownish discharge for several days after abortion and apparently lose the infection within a few weeks (Marsh, 1956).

PATHOLOGICAL CHARACTERISTICS

(1) Foetus: In the absence of putrefactive changes, post-mortem examination of the foetus shows a sanguineous oedema of the subcutaneous tissue particularly of the abdominal wall and blood tinged fluid in the abdominal and thoracic cavities. Petechial haemorrhages are often present in the serous membranes, particularly the epicardium, and subcapsular haemorrhages in the kidneys. The liver may be enlarged and have a number of pale necrotic foci, varying in diameter from 1 mm. to several cm., not unlike the lesions caused by Fusiformis and Histomonas. The stomach contents are usually reddish, mucoid and flaky (Biswal et al., 1953; Tunnicliff & Marsh, 1954; Baker & Stone, 1939; Hartley, 1958; and Miller, 1958).

(2) Foetal membranes: The chorion has many foci of oedema and in severe cases a gelatinous-like material is present in the intercotyledonary areas. Hyperaemia of the placenta is prominent and there may be haemorrhages in the intercotyledonary areas and also within the cotyledons. The cotyledons are deeply congested, soft and pulpy and separate readily. Cotyledons that begin to separate in utero are abnormally pale and often are yellowy-grey in colour. The placenta usually shows marked decomposition. The lesions are not specific for vibriosis and resemble those associated with other uterine infections (M'Fadyean & Stockman, 1915; Biswal et al., 1953; Miller, 1958; Gibert, 1958; Hartley, 1958; and Jensen et al., 1961). Demonstration of V. fetus in stained smears from the cotyledons is a simple and accurate method of diagnosis (Hartley, 1958; and Jensen et al., 1961).

(3) Uterus: M'Fadyean & Stockman (1913) state that the uterus may show no alteration externally but in some cases it is considerably thickened and oedematous in the posterior third. The mucous membrane may be normal in appearance in the early stages but reddened with occasional areas of intense congestion in the submucosal tissue in the more advanced cases. In early cases there is a small amount of grey mucoid exudate and in the later stages a considerable amount of dark reddish mucoid exudate between the chorion and the endometrium. Sometimes a large amount of red watery fluid is also present. The exudate is odourless in uncomplicated cases. Carpenter (1920) reported that the uterine lesions consisted of oedema, congestion and necrosis of the cotyledons. The recently published findings of Jensen et al. (1961) are in agreement with the gross changes described by M'Fadyean & Stockman (1913).

Uteri from ewes which died from vibriosis contained macerated foetuses. Metritis resulted with foci of necrosis leading to perimetritis and peritonitis and, in some cases, discharge of uterine contents into the peritoneal cavity (Jensen et al., 1961).

(4) Histopathology: Jensen et al. (1961) recently published a detailed description of the gross and microscopic lesions of the placenta of sheep affected with vibriosis. They proposed the term "hilar zone" for that portion of the placentome nearest to the hilus of the cotyledon as this is the site of the important changes in vibriosis.

Histopathologically, the changes are concentrated in the hilar zone of the placentome in early vibriosis. In multiple foci the endometrial septa, especially towards the extremities, become necrotic and infiltrated with leucocytes. V. fetus is abundant in the haematomata of the lacunae. The adjacent endometrium shows pericotyledonary inflammation. The uterine glands contain exudate and the endometrium is infiltrated with polymorphonuclear leucocytes. The myometrium is moderately oedematous.

In the advanced stages of vibriosis, the placenta shows arteriolitis in some of the endometrial septa. The endothelial cells are necrotic and degenerated and leucocytes infiltrate the vessel wall, leading to thrombosis. Both arterioles and arterial capillaries are affected. V. fetus is found in abundance in many epithelial cells of the chorionic villi. These parasitized cells are enlarged and unevenly distributed throughout the hilar zone. As abortion becomes imminent, much of the chorionic epithelium of the placentome may become necrotic and detached from the basement membrane of villi, leading to the commencement of separation of the chorion from the endometrium. Chorionic epithelium of the villi desquamates and accumulates in the space between the endometrial septa and chorionic villi. V. fetus penetrates the adjacent mesodermal portions of the villi. Capillaries, either intraepithelial or subepithelial, contain colonies of V. fetus. Some of the endothelial cells appear to be penetrated and the lumen of the vessel becomes partly or completely occluded on account of resulting thrombosis. In areas of heavy infection, V. fetus also invades the chorionic connective tissue. Oedema is extensive in the chorion at the cotyledonary hilus and in many areas, the myometrium is severely oedematous.

IMMUNITY

Very little work has been done on immunity to V. fetus infection in ewes. The consensus of opinion is that the disease is self-limiting as outbreaks of abortion are confined to one lambing season. This failure of recurrence may result from acquired immunity established by the disease or from lack of exposure.

M'Fadyean & Stockman (1913) found little protection when they endeavoured to immunize six ewes by subcutaneous injection of living V. fetus, two months before mating. They stated that these observations should be continued and extended. Jensen et al. (1957), however, presented evidence to show that the self-limitation of the disease, was due to immunity and that it can develop independently of pregnancy and abortion. They found that 44 ewes which aborted from vibriosis during the previous year, and 44 ewes that had been inoculated orally with infected lamb tissues the previous summer as non-pregnant yearlings, were immune when challenged orally with infected lamb tissues during the fifth month of pregnancy. The authors concluded that both the occurrence of vibriosis and oral inoculation of V. fetus-infected lamb tissue, induced immunity in ewes for one year at least.

This suggested the possibility of artificial immunization. Four vaccines, viable V. fetus propagated in egg, viable cultures of recently isolated V. fetus, formolized culture of V. fetus and formolized infected lamb tissues, were prepared. Five ml. of each vaccine was administered parenterally to the ewes of an experimental flock prior to the breeding season. Miller et al. (1958) found no significant difference from the unvaccinated ewes when they were all challenged orally with V. fetus-infected lamb tissues at the beginning of the fifth month of pregnancy. However, a recent report from New Zealand is more promising. Ewes vaccinated with a V. fetus killed vaccine plus adjuvant two weeks before and again nine and a half weeks after mating were later challenged with living cultures. The vaccinates had a 7.5% incidence of abortion compared with 46% of the unvaccinated control group, suggesting that vaccination had afforded a significant degree of protection (Anon., 1959; Boyes, 1959). Later studies to simplify the vaccination procedure did not give clear-cut results and further work is planned to determine optimal dose rates and time of vaccination (Anon., 1960).

Continuing their studies, the Colorado workers induced immunity in ewes with both live V. fetus and formalin-killed V. fetus, administered in two subcutaneous injections prior to breeding. The immunity of the ewes was challenged by one of two methods at the beginning of the last month of gestation; first, by viable cultures of V. fetus per os and secondly, by cohabitation with ewes which had been experimentally infected and were aborting. No abortions occurred in the ewes given viable V. fetus as the immunizing agent. Of the ewes immunized with formalin-killed V. fetus 28% aborted when challenged by oral inoculation and 4% when challenged by cohabitation. Of the control ewes 84% aborted when challenged by oral inoculation and 38% by cohabitation (Miller & Jensen, 1961).

DIAGNOSIS

Diagnosis of ovine vibriosis is based on the occurrence of abortion, and on examination of foetuses, foetal membranes, vaginal discharges and blood samples from aborting ewes. The most reliable method of diagnosis is the demonstration of V. fetus. This may be accomplished by microscopic examination of smears or by culture from the foetus, the cotyledon or the vaginal discharge of the aborting ewe. If the foetus is submitted to the laboratory in good condition, the organism may be obtained in cultures from the stomach, liver or heart blood (Tunnicliff & Marsh, 1954). A tentative diagnosis may be based on the history, the appearance of the aborted foetus and foetal membranes and the distinctive necrotic foci of the liver when they occur. The diagnosis may be confirmed in the field by the direct demonstration of typical V. fetus in stained smears of the foetal stomach contents, liver lesions, affected pale cotyledons and uterine discharges. The specimens to be forwarded to the laboratory should include a whole foetus (preferably several) or sealed pipettes of stomach contents, liver and heart blood; together with portions of the infected areas of the foetal membranes and smears from the infected cotyledons. Such specimens should be dispatched as quickly as possible because of the low viability of V. fetus and to reduce the degree of contamination.

The use of the fluorescent antibody technique should be of diagnostic value in the rapid identification of V. fetus.

The serum agglutination test is considered to be of limited value in the routine diagnosis of ovine vibriosis because of the existence of several serological types and the rapid decrease in serum titres following abortion (Lee & Scrivner, 1941; Blakemore & Gledhill, 1946; Levi, 1950, Hoff & Kaldahl, 1954; and Marsh & Tunnicliff, 1955). As an aid to diagnosis, Tunnicliff & Marsh (1954) suggest that blood samples from several ewes that have aborted recently, be tested for agglutinins.

The recording of positive vaginal mucus agglutination reactions from aborting ewes in a flock infected with vibriosis by Lawson (1959) suggests the possibility that this test may be of value in diagnosis. Likewise, the indirect vaginal mucus haemagglutination test described by Te Punga (1958) may be of value as it is claimed to be more sensitive and accurate than bacterial agglutination.

Ovine vibriosis should be carefully differentiated from other diseases causing abortion such as enzootic virus abortion, brucellosis, salmonellosis, toxoplasmosis and listeriosis.

TREATMENT

There are few reports on the treatment of vibriosis in ewes. Ovine strains of V. fetus, like bovine strains, have been shown to be sensitive to many of the antibiotics in vitro (Prier, 1951 and Ryff & Breen, 1956). Slavin & Weddell (1944) found no effect on the abortion rate or on the recovery rate of V. fetus when 11 pregnant ewes that were inoculated intravenously with cultures of V. fetus, were treated for four days with sulphapyridine. Ryff & Breen (1956) found that single doses of

tetracycline hydrochloride, oxytetracycline and streptomycin failed to influence the abortion rate. Eide & Helle (1957) treated ten pregnant ewes selected at random from a flock which suffered a 12% abortion rate. Two grammes of dihydrostreptomycin were given to each ewe intramuscularly on the first, third and fourth days after isolation. One ewe aborted twins 28 days after the last injection and V. fetus was not isolated from the foetuses. The remaining nine ewes lambed normally. Frank et al. (1958) reported that feeding chlortetracycline at the rate of 80 mg. per ewe daily for 11 days before exposure until termination of pregnancy was relatively effective as a preventive of vibrionic abortion. These workers (1959) also found that two consecutive daily injections of 800,000 units of procaine penicillin G and 1 g. of dihydrostreptomycin sulphate accompanied by chlortetracycline feeding (80 mg. per ewe daily) was effective in experimental groups of sheep. They concluded that antibiotics may be of value in a flock infected with vibriosis. Hulet et al. (1960) observed the effect of treatment in reducing losses in two large flocks. The percentage of treated ewes having at least one live lamb was 86–95% compared to 76–78% in the untreated group. Two intramuscular injections on consecutive days of 800,000 units of procaine penicillin and 1 g. streptomycin was the most effective of the treatments used (91–95%). Chlortetracycline hydrochloride fed at a rate of 80 mg. per ewe daily was the least effective (86%) and did not enhance the efficacy of penicillin and streptomycin when used in combination.

CONTROL

There are no specific control measures described for ovine vibriosis because of our lack of knowledge of the epidemiology. Since the infection is spread primarily at lambing, general sanitary precautions are recommended (Tunnicliff & Marsh, 1954). Aborting ewes should be isolated for two to four weeks until all discharges have ceased. All foetuses and foetal membranes should be destroyed. Disinfection of the infected pen or area is desirable after an abortion, if possible. It must be stressed that infected fluids and tissues be kept away from the feed and water supply of the pregnant ewes (Tunnicliff & Marsh, 1954; Eide & Helle, 1957).

There is no advantage in disposing of ewes that have been through an outbreak of abortion as they will lamb normally in the next breeding season. It is undesirable that sheep from infected flocks be sold for breeding as there is evidence that some may be carriers, nor is it advisable to introduce clean animals into such flocks (Eide & Helle, 1958). These flocks may be regarded as immune to vibriosis and should be kept "closed". The best prevention at present is self-contained flocks, the only introductions being sexually immature animals.

The results of vaccination studies indicate that this procedure may soon be of value in endemic areas. More work is necessary to supplement our current meagre knowledge of the epidemiology of vibrionic abortion before a more practical method of control may be formulated.

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The Abomasum

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In the many reviews of ruminant digestive physiology which have appeared in recent years, the main emphasis has been on the physiological and biochemical events which occur in the fore-stomachs. This is perhaps not surprising in view of the wealth of information now available on these unique digestive chambers and because the digestive processes in the more conventional regions of the digestive tract are unlikely to be very different from those which occur in monogastric animals. Nevertheless the highly specialized development of the fore-stomachs has been accompanied by some modification of the digestive processes in the remainder of the tract and it is becoming increasingly evident that more information is required on the secretory, mechanical and absorptive functions of the lower part of the digestive canal for a more complete understanding of the digestive processes in the ruminant. In this review some of the available information on the first typical part of the alimentary tract, the glandular stomach or abomasum, is summarized and integrated, as far as possible, with the events which occur in the fore-stomachs.

THE ADULT RUMINANT

Anatomy. The abomasum constitutes the fourth division of the ruminant stomach and corresponds to the secretory stomach of other animals. It possesses a wide body region and a long narrow pylorus which terminates at the pyloric sphincter. Anteriorly the abomasum is in communication with the omasum through the relatively wide, unguarded, omaso-abomasal orifice.

The glandular lining of the body region is characterized by large folds which represent an eversion of the mucosa into the stomach lumen. These folds, which are relatively constant in number for a given species (Ellenberger, 1884), commence at the omaso-abomasal junction, rapidly attain their maximum depth, which may be one and a half to two inches, and gradually become smaller as they approach the pyloric region. The latter is covered with a smooth mucous membrane thrown into a few small rugae which resemble those of the simple stomach.

The blood supply to the abomasum is derived from the two divisions of the omaso-abomasal artery which itself is derived from the coeliac axis. Immediately before it passes on to the omasum the omaso-abomasal artery divides into two branches, one of which passes beneath the omasum to course along the greater curvature of the abomasum and

the other which follows the lesser curvature. Both these vessels are embedded in omental fat and give off numerous branches to the right and left faces of the abomasum. They continue to the pyloric sphincter where the superior omaso-abomasal artery anastomoses with the right gastric artery and the inferior omaso-abomasal artery with the right gastro-epiploic artery. The abomasum is thus assured of a copious blood supply: a point of some significance in view of the large volume of gastric juice produced by this organ. The abomasal arteries are accompanied by corresponding veins which ultimately drain into the portal system.

Two main nerve trunks derived from the dorsal and ventral vagi pass over the omasum and follow the lesser curvature of the abomasum. Both trunks are embedded in the fat of the lesser omentum and give off branches to each side of the abomasum. The hepatic-duodenal nerve derived from the ventral abdominal vagus runs in the lesser omentum close to the liver and gives off a branch to the pylorus.

The abomasum is the only part of the ruminant stomach which resembles the simple mammalian stomach in possessing both a submucosal and a myenteric nerve plexus (Sokolowa, 1931; Habel, 1956).

Histology. Although no detailed studies appear to have been made of the arrangement of the muscle layers in the abomasum, typical longitudinal, circular and oblique layers are present. The transition from omasum to abomasum is sharply defined by the change from stratified squamous to simple columnar epithelium on the lip separating these chambers. Brugnone (1810) apparently first recognized the glandular nature of the abomasal mucosa and noted the clear demarcation between the peptic gland region, which contains the large mucosal folds, and the relatively smooth pyloric gland area.

The recognition of the cell types present in the abomasal glands dates from the classic research of Heidenhain (1870), who described the peptic cells of the sheep abomasal glands, and from the description by Rollett (1871) of both the parietal or acid-secreting cells and the peptic cells in the abomasum. Although these observers must have been aware of the nature of the pyloric glands, it was some years later that Sewall (1878) and Pauli (1884) described the essentially mucous nature of the pyloric glands in the sheep. Since that time a number of investigators have reported on the glandular structure of the abomasal mucosa (see Plenk, 1932, Hill, 1951; Sommerville, 1956) and have shown that the gastric glands of the ruminant abomasum are similar in all respects to those of monogastric animals. No attention has however been paid to the rate of cell renewal or the secretory cycle in the various cell components of the mucosa, a somewhat disappointing omission in view of the continuous nature of gastric secretion in the ruminant. Since the changes which occur in the mucosa during digestion in the dog and cat have been likened to those caused by mild irritants (Grant et al., 1953), it is possible that the rate of cell replacement in the abomasum may be very high.

In many mammals the gastric glands have been modified in certain areas, particularly at the junction of the oesophagus and stomach, and were originally termed cardiac glands by Edelmann (1869) because of their relation to the cardiac orifice of the stomach. The cardiac glands

have lost the specialized secretory cells of the gastric mucosa proper and possess only simple mucous secretory cells. They must, in fact, be regarded as glands which represent an intermediate phase in the regression of the glandular mucosa and its replacement by a mucous membrane of the oesophageal type (Bensley, 1902). This replacement has proceeded to a considerable extent in some groups of animals so that large areas of the gastric mucosa possess a stratified squamous epithelium similar to that of the oesophageal region. Because of this and because during development such areas have been frequently isolated in special chambers of the stomach they have been interpreted as being of oesophageal origin and erroneously referred to as oesophageal sacs or areas. The fore-stomachs of the ruminant represent such areas of modified gastric glands and it is in keeping with this concept that a transition zone of cardiac glands is to be found at the omaso-abomasal junction (Haane, 1905; Bensley, 1902, Hill, 1951). The existence of cardiac glands in ruminants was disputed in the older literature and this seems to have arisen partly because of the difficulty in deciding whether glands which occurred at the omaso-abomasal junction should be considered as cardiac glands and partly because of the variation in the extent of the region in different animals (Hill, 1951). There now seems to be no doubt that such glands do occur and it is therefore justifiable to accept Bensley's definition that the cardiac glands are modified fundic glands and that the area occupied by them at the omaso-abomasal junction should in fact be termed the cardiac region. As the junction of the oesophagus and reticulo-rumen is also termed the cardia it would be less confusing if the glands were referred to as cardiac abomasal glands. In further support of Bensley's theory is the fact that isolated mucous glands have been observed in the rumen and in the oesophageal groove (Zimmerman & Sol, 1894).

Further modification of the gastric glands occurs at the junction of the body and pyloric regions where there are glands which consist mainly of mucous cells with only an occasional parietal or peptic cell. This narrow area has been termed the intermediate zone by Billenkamp (1929).

The well developed tunica propria and abundant lymphoid tissue in the abomasal mucosa have been described by several workers but it was not until 1923 that one of the most unusual and characteristic cells present in the mucosa was described. In that year Keasbey published a description of cells found in the abomasum which contained eosinophilic globules and which she termed globule leucocytes because of their similarity to cells found in the dog and termed Schollenleukocyte by Weill (1919). Keasbey records that Professor S. H. Gage of the New York State Veterinary College had previously noted these cells in the sheep abomasum and had provided her with material for detailed histological investigation.

The globule leucocytes, although not confined to the abomasal mucosa, are found there in large numbers. They vary in size from 5 to 12 μ and, although generally spherical, may present an irregular appearance when they are insinuated between other cells. The nucleus, which frequently appears degenerate, resembles that of the lymphocyte and is usually eccentrically placed while the cytoplasm contains

eosinophilic material either in the form of one large globule or as a number of smaller globules. The globule leucocytes resemble the so-called Russell body cells (Russell, 1890) with which they may in fact be identical (Kirkman, 1950; Pearse, 1949). Kent (1952) has reviewed the literature on these cells and has described a complete series of intermediate stages from the small lymphocyte to the fully developed globule leucocyte. The younger cells occur most frequently in the tunica propria and migrate to the epithelium where they remain for some time and are eventually extruded into the lumen.

Numerous theories have been advanced as to the nature of the globules in the globule leucocytes and Russell body cells (Michels, 1955, Kent, 1952) but it is only recently that their true nature has been established. Thus Pearse (1949) showed that the Russell bodies in human plasma cells consist of mucoprotein and White (1954), using the fluorescent antibody technique, has recently shown that the Russell bodies found in mice immunized against S. typhi contain the appropriate antibody. It appears therefore that the globule leucocytes may be concerned in some way with the immune response of the animal although Kent (1952) was unable to obtain any conclusive evidence for a relationship between the presence of parasitic worms and globule leucocytes in the abomasum. More recently, however, Sommerville (1956) has shown that globule leucocytes are only present in the abomasum when sheep are infected with parasitic worms and that they are absent in worm-free sheep. It seems possible therefore that the globule leucocyte may be concerned in some way with the body reaction to parasitic infections. Further work on this interesting problem is required.

Feeding habits and the nature of the food which enters the abomasum.

In the monogastric animal food passes, at infrequent intervals, directly into the stomach where it is digested and passed on relatively rapidly to the lower part of the digestive tract. Observations on the eating habits of ruminants have shown, however, that these animals may spend a considerable proportion of each day eating (Tribe, 1955) and therefore the reticulo-rumen always contains a considerable amount of food material. Even under stall-feeding conditions where animals may be fed only twice or three times a day, or under experimental conditions where they may be fed only once a day, the rumen remains comparatively full throughout each 24 hours. Irregular feeding does however produce differences in the fermentation pattern in the rumen and in the secretory behaviour of the abomasum and it is wise therefore not to translate too closely to the grazing animal the findings obtained under these conditions. It is important too to distinguish between a sheep, which spends its life at pasture, and a high yielding dairy cow which receives a high energy diet and may spend much of its life indoors.

The passage of food material through the digestive tract of the ruminant is a relatively slow process and may take a week or longer, most of the delay occurring in the rumen. Several studies have shown that there is a more or less continuous passage of digesta from the rumen to the abomasum although how this movement is controlled is not fully

understood (see Balch, 1958). Hale et al. (1947) have put forward the view that only material which has been fully digested in the rumen can pass from the reticulum to the omasum. There is, however, evidence that undigested residues of concentrates are excreted more rapidly than those of hay so that, even though there tends to be a separation of digesta in the rumen dependent upon the degree of comminution of the food particles, it is quite possible that some of the most recently ingested food might pass to the abomasum. It is nevertheless a fact that very coarse particles are normally absent or uncommon in the abomasal contents, which is perhaps an indication that the material received by the abomasum has been well digested in the rumen. It is now well established that the fermentation processes in the rumen constitute the major digestive activities of the ruminant and that the more orthodox breakdown of food material is of a somewhat secondary nature. Full descriptions of these events have been given in recent publications (Annison & Lewis, 1959; Lewis, 1961).

The extensive bacterial breakdown of food in the rumen and the subsequent synthesis of bacterial and protozoal protein ensures that the abomasum is supplied with a protein diet of comparative uniformity as the composition of bacterial protein, even when obtained under varying conditions, is relatively constant (Holmes et al., 1953). During fermentation bacterial polysaccharides are also formed and polysaccharide granules accumulate in some of the protozoa.

The importance of the rumen micro-organisms depends therefore not only on their fermentative role in the rumen but on the food material derived from their digestion as they are passed on to the lower parts of the digestive tract.

The outflow of digesta from the omasum appears to be continuous over the whole day and dependent upon contractions of the reticulum. Although recent work (Oyaert & Bouckaert, 1961) indicates that a large proportion of the fluid from the reticulum passes unchanged to the abomasum by way of the omasal sulcus, an event first suggested by Wester (1926) and also observed radiologically by Benzie & Phillipson (1957), there is convincing evidence that the omasum does produce a change in the overall composition of the digesta. Aggazzotti (1910) appears to have been the first to suggest that it would be advantageous to the animal if water was absorbed by the omasum on the grounds that excessive dilution of the gastric juice in the abomasum would therefore be avoided. More recent work has shown that this does occur and that the volume of water absorbed by the omasum may equal the volume of gastric juice secreted by the abomasum (Ekman & Sperber, 1952; Masson & Phillipson, 1952; Gray et al., 1954). Considerable absorption of electrolytes also occurs from the omasum (Garton, 1951, Oyaert & Bouckaert, 1961) and from the point of view of abomasal function the most interesting finding is the great reduction which occurs in the bicarbonate concentration (Ekman & Sperber, 1952) since by this means unnecessary neutralization of the gastric juice is avoided. Volatile fatty acids are also absorbed from the omasum (Barcroft et al., 1944).

Movements of the abomasum and the passage of digesta through the omasum.

The body of the abomasum shows little movement apart from an occasional peristaltic wave. More vigorous movements which are seen occasionally are probably caused by contractions of the fore-stomachs, particularly of the reticulum, to which the abomasum is attached anteriorly. The pyloric region on the other hand exhibits strong regular peristaltic movements which travel towards the pyloric sphincter (Schalk & Amadon, 1928; Dukes & Sampson, 1937; Krzywanek & Quast, 1936; Phillipson, 1939; Benzie & Phillipson, 1937).

Of the four chambers of the stomach the abomasum possesses the greatest degree of intrinsic control over its movements. Thus when both abdominal vagi are sectioned the abomasal contractions persist even though the reticulo-rumenal contractions are abolished (Duncan, 1953). The abomasal contractions after total denervation are generally sufficient to empty the organ although this is accomplished more slowly than normally. Duncan (1953) suggests that the rumen and reticulum, representing the fundus of the primitive stomach, have developed their power to respond to extrinsic nervous control at the expense of their inherent capacity for independent activity and since their anatomy does not lend itself to easy emptying, their co-ordinated cyclic activity bears little resemblance to the motility of the simple stomach. This view is supported by the finding that strips of rumen and reticulum only rarely show independent activity when observed in vitro whereas the abomasum readily commences spontaneous activity and contracts at regular intervals (Duncan, 1954).

That the vagi exert some form of control over the abomasal movements is indicated by the occasional finding that the abomasum is unable to empty itself after vagotomy. Mangold & Klein (1927) and Hoflund (1940) have described delayed emptying of the abomasum after vagotomy and attribute this to pyloric stenosis. Duncan (1953) on the other hand, suggests that the delayed emptying is more likely to be caused by a deficiency in the residual motility of the abomasum as the body is usually found to be distended and the pylorus empty after vagotomy. Direct evidence of vagal complicity in the regulation of abomasal movements is that powerful contractions of the abomasum result from stimulation of the vagi in the anaesthetized animal (Dukes & Sampson, 1937; Duncan & Phillipson, 1951) and that central vagal stimulation provoked by insulin hypoglycaemia produces hypermotility of the abomasum (Le Bars et al., 1953; Hill, 1954; Vallenas, 1956).

The mechanisms which regulate the passage of digesta through the reticulo-omasal orifice are not fully understood (see Stevens et al., 1960). Balch (1958) has discussed this problem and states that the process must be efficient and rapid because in a cow eating 20 lb. of dry matter daily about 14 lb. of dry matter, equivalent to four litres of reticular content per hour, might have to pass through the orifice. There appears to be a continuous passage of digesta into the abomasum and the co-ordinated movements of the reticulum and omasum appear to continue night and day irrespective of the behaviour of the animal. Contraction occurs at a

faster rate during eating and this results in a more rapid transfer of digesta to and from the omasum.

The relatively wide unguarded opening between the omasum and the abomasum would appear to lend itself to the regurgitation of acid abomasal contents. There is no evidence to the contrary and the fact that some of the protozoa are killed in the omasum may be explained by acid regurgitation. It is also quite possible that regurgitation of abomasal content occurs in the young ruminant since milk-clot and whey are not infrequently found in the rumen. In the adult however the continuous movement of digesta into the abomasum presumably tends to prevent back-flow to the omasum and since the body of the abomasum is essentially quiescent, any tendency for the contractions of the pyloric region to force digesta back into the omasum would be minimized. The large mucosal folds in the fundic region of the abomasum might also help to prevent regurgitation (Sisson, 1953).

Digesta appear to pass through the abomasum comparatively rapidly and measurements of the volume of material leaving the sheep and goat abomasum have shown that the flow continues throughout each 24 hours (Phillipson, 1952; Hogan & Phillipson, 1960; Singleton, 1961). Singleton (1961) has, however, emphasized the importance of back-flow of digesta from the duodenum to the abomasum and it may be that the return of a proportion of the digesta to the abomasum may render the digestive processes in the abomasum more effective.

An increased flow rate is observed when digesta are collected from a pyloric fistula and not returned to the duodenum (Phillipson, 1952; Hogan & Phillipson, 1960) and it might therefore be inferred that the abomasum is normally emptying at a rate which is below the maximum of which it is capable and that the flow of digesta is subject to some form of control. Wester (1926) considered that the motility of the fore-stomachs and therefore indirectly the flow of digesta was mainly dependent on the 'state' of the abomasum and several later workers have shown that reflex effects on fore-stomach motility can be evoked from the abomasum (Phillipson, 1939; Clark & Weiss, 1952; Titchen, 1958). Ash (1961) has recently suggested that the inflow of digesta into the abomasum, the secretion of acid and the flow of material from the abomasum are integrated and that the abomasum may in fact be the controlling agent. There is thus an increasing amount of evidence which tends to suggest that the abomasum may play a central role in the activities of the ruminant tract. Although this may be so the importance of the duodenum in controlling abomasal activity must not be overlooked. As in other animals, the intraduodenal instillation of acid, fat or protein digestion products is followed by depression or inhibition of abomasal motility and secretion (Singleton, 1951; Hill, unpublished).

The secretion of gastric juice by the abomasum.

Considerable information on the secretory behaviour of the simple stomach can be obtained by the use of a stomach tube or a gastric fistula. The first of these methods is not applicable to the ruminant and the second is only of limited value since under normal circumstances the abomasum

always contains digesta and it is not possible to obtain samples of pure juice. The only method which permits studies of the gastric juice itself is the use of surgically prepared gastric pouches. Details of the preparation of such pouches in the goat and sheep have been described by Hill & Gregory (1951) and some of the difficulties associated with the drainage of such pouches have been discussed by Masson & Phillipson (1952) and by Hill (1960).

As already mentioned the ruminant at pasture normally spends a considerable proportion of each 24 hours eating and there is therefore frequent addition of fresh food material to the reticulo-rumen. The fact that the ruminant stomach is never empty has long been recognized and it is not surprising that the secretion of gastric juice is continuous. Bickel (1905) and Grosser (1905) were able to show this in a goat with a Pavlov pouch and Savich & Tichomirov (1911), Popov (1932), Belgowski (1912) and Espe & Cannon (1937) showed the same to be true for the sheep and the calf.

Whether the continuous secretion was due to the innate ability of the gastric glands to secrete spontaneously or to their constant stimulation by humoral or reflex means was not clear. Hill (1955) investigated this point and showed that after a fast of up to 48 hours the gastric glands were much less active and that when digesta were prevented from entering the abomasum by emptying the reticulo-rumen the secretion of acid gastric juice ceased. It was concluded that the continuous secretion which occurs in ruminants is not due to the innate ability of the gastric glands to secrete spontaneously but to specific stimuli, the most important of which is the continuous passage of digesta from the reticulo-rumen to the abomasum.

There are considerable practical difficulties in investigating the secretory behaviour of the abomasum under grazing conditions and there is as yet no information on this point. When, however, natural grazing conditions are simulated indoors by the frequent feeding of animals there is no obvious correlation between feeding and the secretory activity of the abomasum (Masson & Phillipson, 1952; Hill, 1960; Ash, 1961). There are minor fluctuations in the amount and acidity of the gastric juice produced and estimates of the total volume of secretion produced by the abomasum are about 5.0 litres per 24 hours for a sheep of 40–45 kg. Kuznetsova (1950) has likewise calculated that the total daily volume of gastric juice produced by a 2-year-old heifer was about 30 litres. This relatively large volume of gastric juice is presumably necessary to cope with the continuous flow of digesta into the abomasum.

Bogdanova (1950) investigated abomasal secretion in calves and in the older animals noticed diurnal changes in secretion. She also observed an increase in secretion associated with the time of feeding. A similar event occurs in sheep and this apparent conditioned response may be explained by the finding of Quin & van der Wath (1938) and Dedashev (1959) that the movements of the reticulo-rumen can become conditioned to feeding and that there can be hypermotility of the fore-stomachs and hence increased passage of digesta to the abomasum at the expectation of food (Hill, 1960). The response was not observed when the fore-stomachs were empty of food so it could not have been the result of a conditioned

reflex affecting the gastric glands directly. A seasonal influence on abomasal secretion was reported by Kuznetsova (1950), who observed that in cattle abomasal secretion was maximal during September to November. Krzywanek & Buss (1935) found that the acidity of abomasal content fell to a low level one month before lambing and gradually rose to normal at lambing.

When ruminants are deprived of food for 24 hours the volume and acidity of the gastric juice secreted by an abomasal pouch are reduced and feeding is then followed by an increase in secretion (Bickel, 1905; Grosser, 1905; Belgowski, 1912). Bickel attributed this increase in secretion to reflex mechanisms but Grosser was inclined to the view that mechanical factors were responsible. Although he did not commit himself definitely it may be inferred that he had in mind the increased rate of passage of digesta through the abomasum under these conditions. Belgowski (1912) in a series of experiments on calves with abomasal pouches described the continuous nature of secretion and the secretory response after a fast and concluded that the secretion of juice was closely connected with the reception of food in the stomach. He was unable to decide whether there was any relation between the rate of secretion and the nature of the diet.

Popov (1902) who used sheep with vagally innervated and vagally denervated pouches showed that the first stage of secretion, the cephalic phase, was not so marked as in carnivora and man and concluded that the pyloric phase of secretion was the most important in the ruminant as direct stimulation of the abomasum by the introduction of food through a fistula was effective in provoking secretion.

Recent work (Hill, 1960) in which sheep were sham-fed when the fore-stomachs were free from digesta has also shown that the cephalic phase of secretion is absent in the sheep. Although a considerable secretory response was obtained during vagal stimulation by insulin hypoglycaemia this response was shown to be mainly the result of an increased flow of digesta caused by reticulo-rumenal hypermotility. The persistence of a small secretory response to insulin hypoglycaemia after the increased flow of digesta from the fore-stomachs had been prevented by prior emptying indicated however that some secretory fibres were present in the vagi and it may be that vagal receptors are situated in a region, possibly the reticulo-rumen, where they are not stimulated by sham-feeding.

Popov's work was subsequently developed by other Russian workers who confirmed that abomasal secretion is conditioned mainly by humoro-chemical factors. Krinitsin (1955), however, showed that a denervated pouch, although capable of secreting continuously, secreted at a lower rate by comparison with that before denervation. This has been confirmed by Hill (1960). Krinitsin considered that the vagi fulfil an adaptive and trophic function in that they intensify the metabolic processes in the cells of the abomasal glands so that they function better under the influence of humoral and chemical factors.

Following on from Krinitsin's conclusion that the basic physiological stimuli of the abomasal glands are humoro-chemical, Nikitin (1940) observed the effect of different foods on secretion. He found that the acidity of the juice increased with increasing rates of secretion although

the enzymic concentration behaved in the opposite manner. Kuimov (1952) in similar work on sheep noted differences in the volume of juice secreted when animals were fed different foodstuffs. He reports that maximum volume responses were obtained with freshly collected hay. It is necessary however in studies of this nature that animals should receive each particular diet for a considerable period before actual comparisons are made and this does not appear to have been done in these experiments. When animals are used to receiving both hay and oats there is some evidence that the secretory response after hay is less than after the concentrate. Volatile fatty acids particularly acetic acid, when introduced into the rumen, have a marked stimulative effect on abomasal secretion and since these substances are produced in large quantities during fermentation and are mainly absorbed from the reticulo-rumen it may be that differences in the secretory response to different foods are related to the rate and extent of fermentation in the reticulo-rumen (Hill, 1960). On the other hand, Popov (1932), Russev (1959), and Ash (1959b), have shown that the addition of fatty acid directly to the abomasum stimulates gastric secretion. It may be therefore that the presence of small amounts of volatile fatty acid in the abomasal content is sufficient to stimulate abomasal secretion. A standardized food intake is necessary in any comparison of secretory responses since an increase in food intake causes an increase in acid output (Ash, 1961). Moreover, large unexplained fluctuations in the volume and acidity do occur occasionally in animals maintained on a constant diet.

The general inference from the preceding work is that the paramount factor in the stimulation and regulation of abomasal secretion is the passage of digesta into and through the abomasum and a reasonably close correlation between the increase in flow of digesta and the increase in secretion after feeding has been demonstrated (Hill, 1960). Ash (1961) also found that an increase in the flow of digesta from the reticulo-rumen stimulates the secretion of gastric acid and moreover showed that the rate of passage of the material through the abomasum appears to be the significant factor in determining the pattern of secretion. It is now becoming apparent that, as in the simple stomach, the acidity of the gastric content is of major importance in the regulation of acid secretion.

Composition of abomasal secretion. The earlier workers provide figures for the free and total acidity of the gastric juice collected from gastric pouches and these figures have been used to substantiate the claim that the acidity attained by the gastric juice of the herbivore is less than that of the carnivore (Lenkeit, 1955). Comparison of the acidities of samples of juice obtained from different animals during feeding responses is not, however, a valid procedure particularly when such diverse animals as dogs and ruminants are concerned.

Investigations on the acidity and electrolyte composition of the abomasal juice obtained from gastric pouches have shown that in conformity with the findings in other animals the hydrogen ion concentration increases with increasing rates of secretion. Maximal concentrations

of hydrogen ion have been found to be 124 m-equiv./l. to 140 m-equiv./l. (Ash, 1959a; Hill, unpublished) and although these are a little below the maximum reported for the dog, 153 m-equiv./l., the latter was obtained after the administration of histamine which is a potent stimulant of acid secretion. Comparable data are not available for ruminants. The concentration of chloride, which is the main anion in the gastric juice, remains relatively constant under frequent feeding conditions. The concentration does however increase slightly with increasing rates of secretion. An inverse relationship exists between the concentration of acid and sodium and a direct relationship between potassium and the rate of secretion of abomasal juice (Ash, 1959a; Hill, unpublished).

The main proteolytic enzyme in abomasal juice is pepsin and the abomasum has been used as a source of this enzyme (Northrop, 1933; Bergmann & Fruton, 1941). Direct comparisons with other species are difficult but there appears to be a relatively high concentration of proteolytic enzyme in sheep and goat gastric juice. Gastric lipase is practically absent in ruminants (Haurowitz & Petrou, 1925).

The mucous secretion of the pyloric region of the abomasum is relatively meagre and is neutral or slightly alkaline in reaction. Its physico-chemical properties and protective function appear to be similar to those of non-ruminants (Bucher, 1932).

The function of the abomasum in the adult sheep. The simple stomach functions as a reservoir and as a digestive organ although the extent of proteolytic digestion which occurs in the stomach is not known. While much has been inferred from simplified systems in which pure pepsin has been allowed to act on a pure substrate, in vitro, detailed information on the events which occur in the stomach itself is lacking (see James, 1957). A somewhat similar situation exists with regard to the abomasum where it is generally assumed that the initial stages of protein digestion occur. There is very little evidence on this point.

When a meal is eaten by a monogastric animal the food, which is swallowed in a semi-solid condition, remains in that state for a comparatively long time and is only gradually penetrated by the gastric juice. In the ruminant on the other hand, the digesta which enter the abomasum are already in a finely divided state and are immediately immersed in a relatively large volume of gastric juice. The abomasal content is usually between pH 2.0 to 3.0 and hence conditions are optimal for peptic digestion throughout the day. It is possible that some of the denser particles which enter the abomasum may remain between the folds of mucosa for some time but the passage of digesta in general seems to be quite rapid. Back-flow of digesta from the abomasum (Singleton, 1961) might increase the time during which the food material is subjected to peptic action and it seems too that the first part of the duodenum can be considered essentially as a continuation of the stomach since the duodenal contents, up to the point of entry of the common bile and pancreatic duct, are frequently between pH 2.5 and 3.0.

It is generally assumed that rumen protozoa and bacteria are killed in the acid environment of the abomasum; although this has been shown to

be so for the protozoa, there is no direct evidence that the rumen bacteria are in fact killed in the abomasum (Pounden et al., 1950; Masson, 1950; Weller & Gray, 1954). Cultural studies on the abomasal content are obviously required.

Investigations on the composition of the abomasal digesta have been mainly on material removed from the abomasum after death (see Boyne et al., 1956; Badawy et al., 1958a, b; Gray et al., 1958) and whilst of value for certain purposes they provide no information on the fluctuations which may occur in the normal animal. Many of them too now require revision in the light of recent findings of Badawy et al. (1957) that the nitrogen content of digesta may be considerably increased by shedding of the mucosa when animals are shot. Hogan & Phillipson (1960) have attempted to overcome this problem by permanently exteriorizing the flow of digesta at appropriate points along the intestine. By this means the flow and composition of the digesta can be determined.

There is little information on the absorptive capacities of the abomasum but by analogy with the simple stomach it is probably unlikely that much absorption of the products of food breakdown occurs in this organ. Evidence of slow absorption from the abomasum was obtained by Colin (1873) who placed nux vomica in the tied-off abomasum of a calf and found that the calf showed symptoms of poisoning after 4.5 hours and died after 7 hours. Barcroft et al. (1944) found only traces of volatile fatty acids in the blood draining the abomasum. Lack of absorption of ammonia from the abomasum is indicated by the work of McDonald (1948).

THE YOUNG RUMINANT

Development of the abomasum. As in monogastric animals the stomach develops from the simple spindle-shaped enlargement of the primitive digestive tube (Pernkopf, 1931; Lambert, 1948; Warner, 1958) but because the rumen, reticulum and omasum are lined with statified squamous epithelium there have been repeated claims in the literature that they are in fact derived from the oesophagus. The abomasum itself is derived from what in other species becomes the pyloric antrum while the reticulum and rumen correspond to the fundus and body.

At 39 days (ox) the abomasum differentiates into a wide cranial and a narrow caudal region which are the fore-runners of the body and pyloric regions of the adult abomasum. As development proceeds the longitudinal abomasal axis bends increasingly so that the lesser curvature between the body and pylorus becomes progressively more acute. The inner lining of the abomasum develops a pair of longitudinal folds at 38 days and subsequently the entire mucosal surface of the body region is involved in the folding process until a large number of large and small folds are present. Similar elevations of the mucosa appear in the pyloric area but these are more comparable to the rugae of the simple stomach and do not attain the large size of the folds in the body region.

It is of interest that in foetal life the future large size of the rumen is foreshadowed by a relatively excessive development of the rumen dilatation. Thus up to four months from conception the rumen is two to

three times the weight of the abomasum. After this time the abomasum develops more rapidly than the other compartments so that by six months the abomasum weighs as much as the other three compartments combined (Becker et al., 1951). The accelerated development of the abomasum in relation to the other compartments of the stomach ensures that it is of sufficient size to meet the demands made on it immediately after birth.

The relative proportions and capacities of the rumen and abomasum undergo considerable change after birth; the rumen attaining much larger dimensions than the abomasum as the animal changes over from milk to an adult diet. Wardrop & Coombs (1960) found that during the first week after birth the abomasum had the fastest growth rate of all the parts of the tract but from this time onwards its growth rate slowed down and fell below those of other organs. Tamate (1957) has discussed the striking differences between the post-natal development of the abomasum and reticulo-rumen in the first two months after birth and, on the assumption that the capacities of the rumen and abomasum are direct indications of their digestive functions, suggests that the two organs are in different stages of development, i. e., the large abomasum in new-born animals has practically reached the end of development and the relatively small increase in its capacity in the first two months of life is due to the growth of the animal. On the other hand, the small rumen in the new-born animal represents an immature organ which remains immature until suckling is completed. Tamate also showed a definite correlation between abomasal capacity and the body weight of the animal and suggests that the efficiency of digestion in the rumen largely depends on its volume and that the efficiency of the abomasum depends on its mucosal surface area rather than its capacity.

Recent studies on the growth of the ruminant stomach have followed on from Lagerlöf's (1929) finding that the relative size of the four compartments was determined to some extent by the nature of the diet. Lagerlöf considered that roughage was important in this respect but later work has indicated that the chemical nature of the digesta is of greater significance (Brownlee, 1956; Warner et al., 1956). Sander et al. (1959) have shown, for example, that solutions of fatty acids cause marked development of the rumen mucosa when administered to young calves. Feeding experiments have shown that papillary development of the rumen mucosa is also greater for calves fed dry feeds compared with those which receive milk. The importance of this work in so far as the abomasum is concerned rests on the fact that acceleration of rumen development and function is now possible and hence the young ruminant can be converted to the adult type of digestion much earlier than has been customary. Although there seems to be no a priori reason why abomasal function should not develop in parallel with that of the reticulo-rumen, there is no physiological information on this interesting point.

Histogenesis. Descriptions of the development of the gastric mucosa in the sheep have been provided by Sewall (1878) and Hill (1956) and of the early stages of development in the cow by Warner (1958). In the sheep the primary epithelial lining of the simple stomach spindle consists of a single layer of columnar cells which gradually become folded

and so form projections into the lumen of the stomach (40 days). These projections, consisting of a core of mesoblast covered with columnar cells, eventually form the large mucosal folds of the adult abomasum. In the 60–75 day foetus the columnar epithelial lining of the entire stomach is formed into a series of shallow tubular pits, the future gastric glands, and it is by differentiation from the simple columnar cells which line them that the various cell types of the mature glands are produced. Thus in the slightly older foetus (75–85 days) it is possible to distinguish the future peptic cells as small groups of darkly staining cells at the bases of the glands. By 85 days many of the peptic cells contain poorly defined granules which represent the developing zymogen granules. From this time onwards there is progressive development of the glands which results in an increase in their depth and in the number of peptic cells. The latter contain numerous zymogen granules which can be demonstrated clearly from 119 days onward. The most interesting feature of the developing gastric gland is the scarcity of parietal cells. Thus at 120 days they are very infrequent and even at term (150 days) they are relatively uncommon. This state of affairs is however rectified in the first day or so after birth by a rapid increase in the number of parietal cells (Hill, 1956).

Of considerable interest is the location of the rennin-secreting cells in the mucosa. Although it is many years since it was pointed out that the gastric glands of some animals contain rennet zymogen which must be activated to produce the enzyme rennet the actual location of the rennin secreting cells has not yet been determined. Edkins (1898) has suggested that the peptic cells contain the zymogens of both rennin and pepsin and poses the question as to whether there are different granules for the two enzymes or whether they are contained within the same granules. The situation appears to be analogous to that in the pancreas where the zymogen granules are present in only one kind of cell and where it is legitimate to suppose that all the enzymes are elaborated by the same kind of secretory cell (Babkin, 1950).

Feeding habits and the nature of the food which enters the abomasum.

Observations on the young lamb have shown that suckling may occur as frequently as once hourly up to the age of fourteen days (Munro, 1955). There is however a gradual reduction in the time the ewe allows the lamb to suck and a sudden shortening of this period occurs at approximately 14 days. Lambs start picking at grass before they are 48 hours old but only begin to eat grass between 14 and 18 days. After this time the frequency of suckling drops to about six times daily (Barnicoat et al., 1949). Under pasture grazing conditions cows suckle their calves about three times daily, each suckling period lasting about 15 min. (Johnstone-Wallace & Kennedy, 1944) although it is probable that suckling is more frequent shortly after birth.

The first milk obtained after parturition is colostrum and its composition differs markedly from that of later milk; the most striking difference being in the concentration and distribution of protein. Thus there may be 17–18% protein in the first colostrum, although this falls

rapidly during the first 24 hours after parturition and gradually approaches that found in normal milk. Although the high protein concentration of colostrum is of major significance in the transmission of immunity to the young it is obviously also of considerable nutritional significance.

The milk of ruminants is a typical casein milk, i.e., casein amounts to some 75–85% of the total protein of the milk and as such forms particularly firm curds which tend to remain longer in the stomach than those formed from the so-called albumin milks (Johansson & Claesson, 1957). The whey formed by the removal of casein contains about 20% of the total protein of the milk and has a complex composition containing euglobulin, pseudoglobulin and lactoglobulin and other components. During the process of evacuation of the udder in the cow the percentage of fat present rises, the first portion of the milk removed may contain only 1–2% of fat whereas the last portion may contain 8–15%. Assuming that a similar state of affairs exists during natural suckling this increased fat content towards the end of the meal may slow down gastric emptying through the enterogastrone mechanism. An indication that this may be so is that fat, when included in modified milk diets, reduces the incidence of diarrhoea (Owen et al., 1958). Enzymes, e.g., lipase and protease, are present in milk but whether they fulfil any digestive role is unknown. Mention should be made too of the findings of Wise and his associates (Wise et al., 1940; Ramsey et al., 1960) that secretions from the base of the tongue and pharyngeal end of the oesophagus of the calf contain lipolytic enzymes which may be of importance in the digestion of milk and in accelerating the clotting of milk in the abomasum. No similar studies have been done in the lamb.

The gradual change over from a complete milk diet to the adult diet in the calf has received considerable attention in so far as the changes which occur in the reticulo-rumen are concerned. This work has received impetus because of the economic importance of the early weaning of calves. Little similar work has been done on lambs since it is not common farming practice to remove the lamb from the ewe during the first few weeks of life. There is little doubt that milk and fresh growing grass are ideally suited for the production of rapid growth in lambs and as ewes' milk is of no commercial significance in Gt. Britain there has been no necessity to develop systems of early weaning in this species.

During the weaning period, which has been considered to end by the time the calf is about six weeks and the lamb about three weeks old (Phillipson & Cuthbertson, 1956), the abomasum receives milk directly via the oesophageal groove and fermented material from the reticulo-rumen. There is no information on the relative contributions which each makes to the energy requirements of the animal but estimations of the volatile fatty acid concentration in the rumen contents have shown that although volatile fatty acids appear in the blood and rumen liquor of calves within one week of birth (McCarthy & Kesler, 1956) it is not until calves are about three months old that the rumen volatile fatty acids are comparable to those of the adult (Lengemann & Allen, 1955). This period however must depend very much on the system of feeding and in view of the increasing tendency to reduce the weaning period and to accelerate

rumen development it may be important that attention is also paid to the events in the remainder of the tract. Before the rumen is fully functional some of the solid food material presumably passes directly to the abomasum without the interposition of bacterial action and it would be of interest to know how efficient the digestive processes of the young ruminant are in handling this material.

The passage of food in the young ruminant. The presence of a reflex mechanism whereby the fore-stomachs are by-passed and ingested milk is directed to the abomasum has been known for many years but it is only comparatively recently that detailed information has been obtained on this event (see Comline & Titchen, 1961). From the point of view of the most rapid and efficient digestion of the milk it is important that the groove should function efficiently and it appears that in the calf the most effective way of ensuring this is to allow the calf to suck (Schalk & Amadon, 1928; Wise & Anderson, 1939). Watson (1943) on the other hand concluded that in the lamb the desire of the animal for food was the determining factor in groove closure and that closure could in fact become a conditioned reflex. The groove reflex develops in foetal life (Duncan & Phillipson, 1951), becomes maximal in the very young animal, and is less easily elicited as the animal becomes older.

Correct functioning of this reflex ensures that most of the meal passes directly to the abomasum and it is obviously of great importance to the suckling ruminant since severe gastro-intestinal disturbances occur when there is malfunction of the groove (Wise & Anderson, 1939; Schalk & Amadon, 1928). It is probable nevertheless that some back-flow of milk to the rumen may occur after feeding (Pounden et al., 1955; Benzie & Phillipson, 1957).

There does not appear to be any reliable information on the length of time a single milk meal remains in the abomasum. The milk rapidly separates into clot and whey in the stomach and as the whey appears in the duodenum within five minutes of feeding (Phillipson, 1939) it is evident that the passage of fluid is a rapid process. In practice it is unlikely that the abomasum is ever empty since meals are usually taken at frequent intervals but it is of interest to know something of the gastric emptying time. Mortenson et al. (1935) endeavoured to determine the rate at which milk left the abomasum by placing milk in this organ through the reticulo-omasal opening and palpating the abomasum for the presence of curds. They found that boiled and autoclaved milk (8–12 hours) was usually evacuated faster than raw milk (12–18 hours). Espe & Cannon (1935) used the same technique and found that milk which contained up to 6% fat tended to leave the stomach more rapidly than skim milk. They attributed the increased emptying time to differences in the texture of the curd produced when fat was present in the diet. In all this work the method of introduction of the milk prevented its admixture with pregastric lipase (Wise et al., 1940) and may not have stimulated the secretion of gastric juice to the same extent as normal sucking. Both of these events may influence the rate of breakdown of milk clot in the abomasum and as the act of sucking also stimulates the movement of the abomasum (Phillipson,

1939) the emptying time may have been longer than normal.

Secretory and digestive processes in the abomasum of the young ruminant.

An understanding of the enzymic changes which occur during the development and growth of the digestive tract is of particular importance in food-producing animals and it is to be regretted that so little information is available on the young ruminant.

The early investigations on this topic were almost entirely concerned with the analysis of tissue extracts and have been discussed in detail by Needham (1931). Much of this work now needs to be repeated with modern methods and extended to include the analysis of the digestive juices obtained from the living animal since examination of tissue extracts does not necessarily indicate which enzymes are present in the actual digestive secretions and does not reveal the changes which might occur under different feeding conditions. Some of the limitations of this kind of work are evident from the investigations of Walker (1959) who has shown that in young lambs estimates of the proteolytic activity of abomasal content do not give any real indication of the proteolytic activity of the gastric secretion and that extracts of abomasal wall are of little value in determining changes in the enzymic properties of the stomach.

The deficiency of acid-secreting cells in the abomasal mucosa of the new-born lamb is reflected in the composition of the abomasal secretion. Thus Phillipson (1955) and Hill (1956) have shown that the reaction of the abomasal content of the lamb at term is near neutrality. After birth the parietal cells develop rapidly and are accompanied by a corresponding fall in the pH of the abomasal contents so that by some 36 hours after birth this is about pH 3.0. Since maximal proteolysis by the abomasal content of young lambs when tested in vitro occurs at pH 2.1 it seems reasonable to assume that the young lamb secretes pepsin and that maximum proteolysis in vivo does not occur until the lamb is about 36 hours old. This lag period in the onset of optimal proteolysis, which corresponds with the period when colostral proteins are absorbed, may represent a mechanism whereby the antibodies are protected from digestion as they pass through the abomasum (Hill, 1956). Whether rennin is secreted by the young lamb has not been satisfactorily determined but it is a fact that the colostrum sucked immediately after birth is clotted very efficiently, indicating that perhaps rennin might be secreted by the lamb. It is however true that all proteolytic enzymes are able to clot milk and although the pH of the abomasal content of the new-born lamb is not suitable for proteolytic digestion by pepsin it is eminently suitable for the coagulation of milk by this enzyme as this reaction is optimal at pH 5.25 (Lundsteen. 1934).

One of the most characteristic features of the young calf is the marked milk coagulating power of its gastric juice and of extracts of abomasal mucosa; properties which have long been utilized for cheese making. There has been a protracted controversy as to whether milk coagulation is caused by a distinct enzyme, rennin, or whether it is the result of peptic activity and it is only comparatively recently that the question has been finally resolved by the preparation of crystalline rennin (Hankinson, 1943; Berridge, 1945). Fenger (1923) suggested that rennin

was the only enzyme present in the calf stomach, but Fomin (1939) appears to have been the first to show that rennin is present in the gastric juice of the calf and is secreted during feeding. The relationship between pepsin and rennin in the gastric juice of the calf has not been completely elucidated but there are some indications that rennin is the only enzyme present at birth and that provided the animal is maintained on a pure milk diet only rennin is secreted. Berridge et al. (1943) found that in calves maintained on a milk diet there was a high rennin to pepsin ratio but as the proteolytic activity was optimal at pH 3.0 to 4.0 it is quite possible that this was attributable to rennin and not pepsin. Although it has been generally considered that rennin has only a weak proteolytic action (Sumner & Somers, 1947; Nitschmann & Varin, 1951), Fish (1957) has shown that rennin is, in fact, a powerful proteolytic enzyme with a pH optimum of 4.0. There is no information on the acidity of the abomasal content or gastric juice of normal calves during the early days of life but Berridge et al. (1943) found that in calves maintained on a milk diet the acidity was low during the first few weeks of life. In other words, the conditions in the abomasum were optimal for the clotting and proteolytic activities of rennin and it was not until solid food was introduced into the diet that the acidity increased rapidly.

In dogs and man the ratio of milk clotting activity to proteolytic activity of the gastric juice is apparently independent of the age of the animal, that is, both activities are attributable to pepsin (Holter & Andersen, 1934). This is not so in the young calf where there is a very high milk-clotting to proteolytic ratio. During the weaning of the calf the proteolytic activity of the abomasal secretion gradually increases, presumably as a result of the secretion of pepsin, although the clotting activity remains unchanged. This might seem somewhat paradoxical if the rennin content of the juice diminishes and the peptic content rises. The clotting activity of pepsin may however contribute to the total clotting activity observed and it is only at a much later stage that the proteolytic (peptic) activity to clotting (peptic) ratio is much greater. Holter (1932) showed that in the adult cow pepsin was the proteolytic enzyme and rennin was no longer present.

The change over from rennin secretion to pepsin secretion by the abomasal glands is an intriguing one and merits closer study with modern techniques. Oppenheimer (1926) has suggested that there is a gradual change from the production of rennin to the production of pepsin by the gastric glands and as it is now possible to distinguish the two enzymes electrophoretically (Fish, 1957), it should be possible to follow this change in the juice of calves with abomasal pouches.

Grosskopf (1959) has attempted to do something along these lines by examining the clotting and proteolytic activity of gastric juice obtained from calves with abomasal pouches but as the calves were receiving only milk it is probable that the proteolytic activities of the juice were attributable mainly, if not entirely, to rennin. This is borne out by the similarity in the rates of secretion of the clotting and proteolytic agents. Drinking milk increased clotting and proteolytic activity, the maximal effect being reached 10–20 min. after the start of feeding. It is unfortunate that the acidity of the gastric juice was not determined.

Fomin (1939) developed a method for the production of rennin in which young calves with abomasal fistulae were fed milk and the abomasal content then collected for the preparation of rennin. Berridge et al. (1943) repeated this work but found that the yields of rennin were so low that the method was not economical. By feeding whey slowly and collecting it as it emerged from the abomasal fistula they found that there was a lag of about 5 min. between the start of feeding and maximum secretion of rennin.

The mechanisms which control the secretion of gastric juice in the young ruminant have not been fully investigated. As suckling occurs at frequent intervals it is improbable that the abomasum is ever entirely free from food material and it is likely therefore that the secretion of gastric juice is continuous and analogous to the adult ruminant. Espe & Cannon (1937) prepared Pavlov pouches in calves and from the lack of response of the pouches to 'sham-feeding' they concluded that the psychic or cephalic phase was of little importance to these animals. Skulov (1950) on the other hand reported that sham-feeding of oesophagotomized calves for 5–10 min. stimulated gastric secretion. Shoptaw et al. (1937) also prepared gastric pouches in calves and compared the effect of feeding whole milk and soya bean gruel on the secretory activity of the pouch. Both foods produced a secretory response which was maximal 60–90 min. after feeding and subsided over the subsequent 90 min. to pre-feeding levels. This type of response compares with that seen after feeding adult sheep and is doubtless mainly attributable to the pyloric phase of secretion. Secretion continued at a lower level after the secretory response had subsided and presumably represented the intestinal phase of secretion. Estimations of the free and total acidity of the abomasal content were made in some experiments and, as expected, the amount of free-acid present was small; total acidity was maximal 3–4 hours after feeding.

The mechanisms controlling the secretion of rennin in the gastric juice of the calf are not known but the rapid increase of this enzyme after feeding is indicative of a neural, presumably vagal, mechanism. There is no information on the secretion of pepsin by the young lamb but it would not be unreasonable to expect similar vagal control and a rapid increase after feeding because in both the sucking calf and the lamb the milk passes directly to the abomasum and the situation is analogous to that in the monogastric animal where there is a sudden outpouring of gastric juice at the beginning of a meal. In the adult ruminant because of the continuous flow of digesta there is no necessity for a sudden increase in secretion after feeding and this is presumably the reason why vagal stimulation is of little significance. It is perhaps in keeping with this idea of a decrease in vagal activity as the ruminant matures that differences in the secretion of pepsin by adult sheep might be attributable to individual differences in the degree of loss of vagal participation in the secretory process.

The changes which occur in the abomasal secretion of lambs during growth have been investigated by Kuimov (1956), who found that the daily secretion of abomasal juice increased 15–24 fold between the ages of 1–6 months reaching 4.5–6.5 litres in lambs 6 months old. The proteolytic activity of the juice declined after the animals were six weeks old and then

gradually increased again. The reduction in activity was associated with the introduction of large quantities of plant food into the diet and Kuimov emphasizes the necessity for making the change from milk to a vegetable diet a gradual one. The volume and acidity of the abomasal secretion of the calf also increases during growth (Bogdanova, 1950).

Colostrum and milk are particularly suitable for digestion in the somewhat immature digestive tract of the young ruminant where the acidity is low and enzymic development may still be taking place. Ruminant milk produces a heavy dense clot which is mainly due to the relative proportions of globulin and casein in the milk, a higher proportion of the former inhibiting clotting while the latter favours it. Colostrum, because of its high globulin content, produces an open, readily digestible clot, but with the progressive reduction in protein the clot tends to become denser. Infrequent large feeds of milk are therefore contra-indicated since they may result in the production of excessive amounts of cheese-like clot which accumulates in the abomasum and may lead to digestive disturbances (Sheehy, 1934). Excessive filling of the abomasum and reticulo-rumen also occurs when single large meals are taken by calves and the resultant massive clot formation, it was suggested by Wise et al. (1940), interferes with the digestion of the chyme which left the abomasum.

The actual process of clotting in the abomasum does not appear to have been investigated in detail although there are many studies on the clotting mechanism and the hydrolysis of casein in vitro (Christensen, 1954; Cerbulis et al., 1960). For the animal on a milk diet adequate clot formation in the abomasum is of great importance in the regulation of the flow of material from the abomasum, for if clot formation is inhibited, then scouring inevitably results (Kastelic et al., 1950; Blaxter & Wood, 1953). This finding is pertinent to the problem of devising synthetic diets for calves where vegetable protein which forms the main protein source is not coagulated in the abomasum. As Preston (1957) has pointed out, the calf is dependent on rennin during its early life for the initial stages of protein breakdown and as this enzyme is to some extent specific for casein there is little to be gained in introducing alternate sources of protein in the diet of very young calves. The alternative approach, to induce adult-type rumen function at an early age, appears to be a more logical and successful method of weaning young calves (Preston, 1956, 1957, 1958).

CONCLUSIONS

The rapid increase in knowledge of the events which occur in the rumen has not been accompanied by similar advances in our understanding of the eventual fate of the rumen digesta and the manner in which they are digested. Although only the abomasum has been discussed here, the information on pancreatic, bile and intestinal secretion is even less complete and there is need for a systematic study of the digestive glands and their secretions in the ruminant.

Continuous secretory activity by any gland is an interesting phenomenon and its morphological basis in the abomasum merits detailed

investigation. This may well have some bearing on the composition of the abomasal content as cell turnover rates must be high and desquamation of dead cells appreciable. Detailed information on the composition of the abomasal secretion, particularly its enzyme content, is relatively meagre and there is even less known about the overall composition of the abomasal content and the effect of gastric digestion on its various components. Variations in diet, such as the change over from dry to pasture feeding, produce rapid alterations in rumen activity and some attempt should be made to correlate these changes with secretory activities, particularly in relation to metabolic upsets where modifications in secretory behaviour may affect the absorption of electrolytes.

Quantitative measurements of the flow of digesta along the digestive tract are difficult to make and the attempts which have been made so far, although providing some approximate values, have tended mainly to underline the problems which still require solution. Continued attempts in this direction are certainly necessary for more precise estimates of the relative importance of ruminal and intestinal digestion and the rate of passage of dietary components.

The recent upsurge of interest in the young pre-ruminant animal and the demonstration that the development of the digestive tract can be controlled, to some extent, by manipulation of the diet, has opened up a new field of investigation which is still in its infancy. As in the adult ruminant an understanding of the secretory events in the developing tract has lagged behind that of the changes which occur in the fore-stomachs of the young ruminant and systematic studies are required of these, particularly in the calf. The clotting and digestion of milk itself has not been fully investigated and would seem an obvious starting point for an understanding of digestion in the young animal. Investigations on gastro-intestinal upsets, which occur frequently in young animals, have been relatively few and their underlying causes are only vaguely understood. A more intimate knowledge of the physiological happenings in the gastro-intestinal tract must precede an informed understanding of the aberrations produced during disease.

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